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INTERLEUKIN 2 RECEPTOR (sIL-2R), AND THEIR CORRELATION WITH
AGE, GENDER AND THE ONSET OF CHILDHOOD ATOPY

by

Alice Lorraine Miller

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ABSTRACT

Identifying predictive indicators of atopy could allow for the early intervention and/or prevention of atopic associated diseases within individuals identified to be at high risk. Two immunological factors advanced as candidates for such a role are sCD23 and sCD25. Soluble CD23 and CD25 expression were therefore examined in the cord blood of over 300 healthy newborns enrolled in the Tucson Children's Respiratory study. Additionally these factors were measured in sera drawn from these patients between the ages of 4-9. Determinations were made using commercially available sCD23 and sCD25 ELISA assays. Results indicate these markers are measurable in cord samples, they are not influenced by gender, and soluble CD23 and CD25 levels decrease with age. Increasing levels of sCD23 and sCD25 did not correlate with increasing expression of IgE. Experimental data derived in this study indicate these factors will not serve as independent, predictive indicators of future asthma, hayfever or eczema.

INTRODUCTION:

Atopy can be defined as the predisposition to respond to environmental allergens with the production of specific IgE. Asthma, a reversible obstructive airway/bronchial disease, is often associated with atopy in children. (5) Increased levels of IgE is the common thread tying together the atopic associated conditions of asthma, allergic rhinitis and atopic dermatitis. Skin testing for allergens in childhood asthmatics reinforces the fact that there is a close association between asthma and atopy. (43) It is a matter of record that eighty-five percent of childhood asthmatics test positive for one or more skin test allergens. (43) The transition from childhood to adulthood asthma is also strongly associated with atopy and persistent airway hypersensiveness. (43)

Epidemiologic trends reveal the primary cause of absenteeism in school age children is asthma. Nationally, 7.6% of children ages 6-11 have had asthma; worldwide rates vary and can reach as high as 17% as it is in New South Wales, Australia. (43) In a Tucson study, the prevalence of asthma in children by 4-10 years of age was 13%. (unpublished) Additionally over the past 25 years hospital admissions for childhood asthmatics under the age of 15 have increased

dramatically despite decreasing admissions for the overall category of respiratory illnesses. (43)

Possessing predictive indicators for atopy very early in life could therefore have very important clinical implications for the intervention, prevention and/or early treatment of future atopic disease.

Serum IgE levels are often increased in allergic individuals. (2, 25) In a 1991 study Matsumoto reported increased levels of interleukin-4 (IL-4) in all allergic groups. (28) B-lymphocytes in atopic subjects are known to secrete IgE in response to airborne allergens and other antigens. Additionally the proportion of low affinity receptor for IgE (FCεRII, or CD23) (+) lymphocytes has been shown to be increased relative to normal in allergic subjects and in those individuals with non-allergic hyper-IgE conditions. (13, 20, 36). Soluble CD23 (sCD23) is also found in large quantities in the circulation of atopic subjects evaluated. (46, 50) These facts lead one to suspect that FCεRII(+) lymphocytes participate in the regulation of in vivo IgE synthesis by the production of soluble FCεRII. (41) IL-4 treated cells not only express more CD23 on their surface but they also release more sCD23 in turn. (13)

It would seem that the best predictor of future atopy would be obtained by simply measuring IgE levels at birth. Using Prist RIA assays, US and European studies reveal infants in developed countries are not routinely stimulated to produce IgE in utero. (15, 16) Approximately 55% of newborn blood evaluated contained detectable levels of IgE. (15) Undetectable IgE levels are considered those below 0.1 IU/ml. Newborns possessing high levels of IgE in their cord samples did not yield a higher percentage of children medically diagnosed with asthma at age 6 as compared with the group of neonates with values less than 0.1. (personal communication with Debbie Stern) IgE values obtained from specimens drawn from infants during well-baby check-ups (9 months) however are predictive of asthma which develops by age 6. (personal communication with Debbie Stern) It appears the immune factors which provide the capacity to develop high IgE levels occur after birth. Correlation studies of IgE levels at birth with the development of asthma have remained inconclusive.

Asthma and allergic rhinitis are both characterized by mucosal inflammation. CD4+ T lymphocytes as well as eosinophils and mast cells are particularly abundant at these inflammatory sites. The association of T lymphocytes with atopic inflammation has led numerous researchers to investi-

gate the regulatory role of T lymphocytes in the expression of atopy. (19)

Physical interaction between T cells and B cells and at least two sets of signals are required for IgE synthesis. (3, 9, 34, 38, 48) Derived from TH2-Lymphocytes or mast cells, the interleukin-4 is considered to play an essential role in the events which drive uncommitted B cells toward IgE presentation in vitro and in vivo. (13, 31, 33, 35, 34, 38, 42, 48) IL-4 provides the crucial signal for initiation of germ-line transcription through the epsilon (ϵ) locus; however additional stimuli are required for the expression of mature ϵ mRNA and IgE synthesis by B cells. (25, 33, 38, 44, 48) The second set of signals can be provided by CD4+ T cells expressing CD23 after cognitive interaction between TCR/CD3 complex and MHC Class II antigens, B-cell activators including Epstein Bar Virus, hydrocortisone and/or monoclonal antibodies (mAb) to CD40. (25, 35, 44, 48) Expression of CD23 on B cells, monocytes and T cells is potentiated by IL-4. (3, 33, 38) Refer to figure 1.

In humans, CD23 appears as an early, stage-specific marker in the ontogeny of the IgM-bearing B cell. Experimental studies using mAb to CD23 have demonstrated that greater

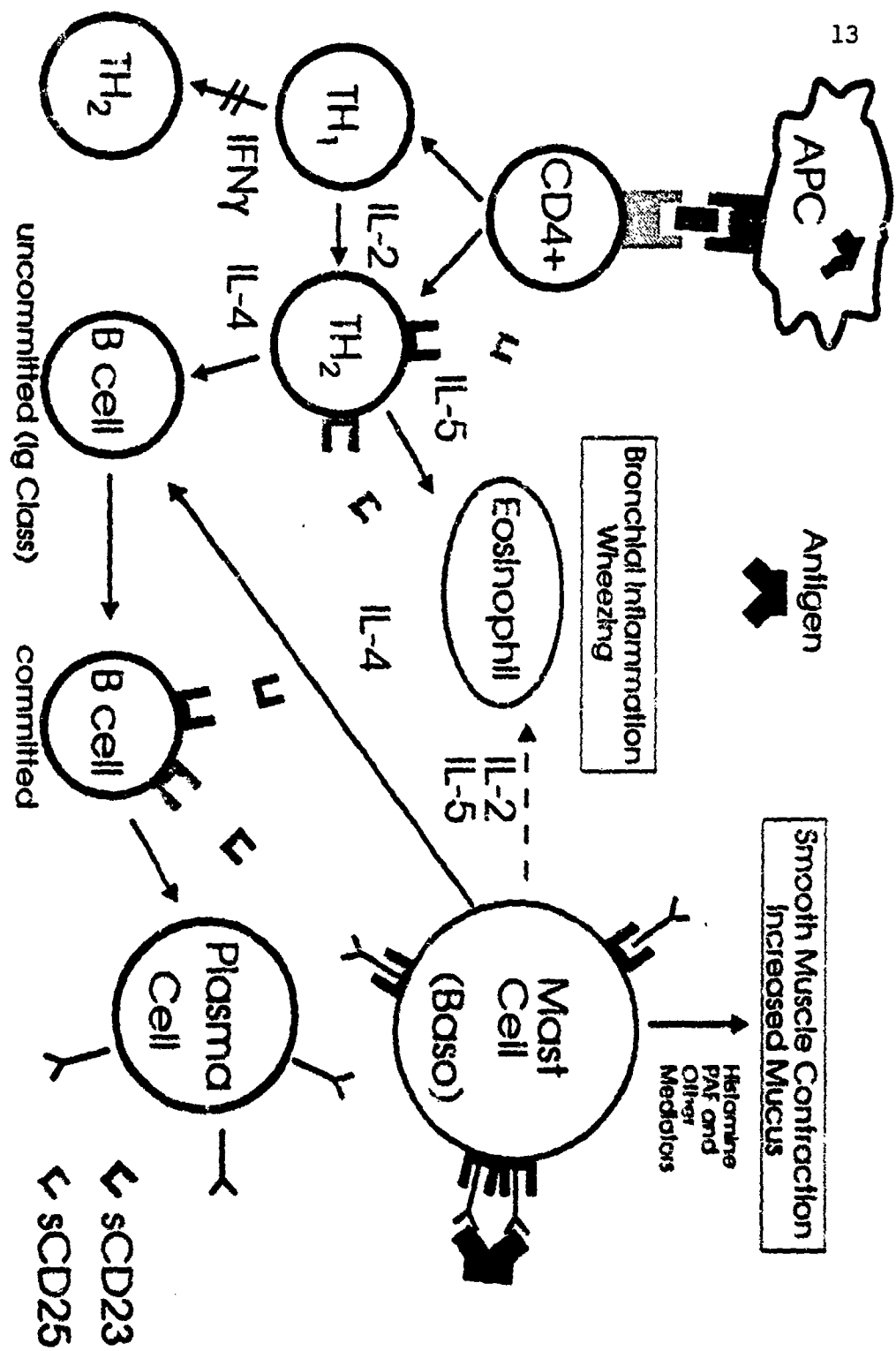


FIGURE 1. Schematic for IgE synthesis. A hypothetical model of Immunological mechanisms for the production of IgE.

than 90% of sIgM+ and sIgD+ B lymphocytes constitutively express CD23. (14, 20, 51) IL-4 not only serves to increase the density of CD23 on the surface of sIgM+/sIgD+ B-cell surface but also induces CD23 type B expression. (1, 3, 7, 8, 9, 11, 20, 51) When CD23 is up-regulated it is accompanied by receptor associated autoproteolytic cleavage and release of IgE potentiating factor (sCD23) from the membrane bound CD23. (2, 4, 7, 34) The sCD23 triggers the growth and differentiation of B-cells, and is a cofactor in the induction of the IgE response by IL-4. (20, 37, 39) By up-regulating CD23, IL-4 serves to maintain a steady-state level of IgE. (6, 11) Modulation of CD23 is regulated by the release of other lymphokines such as IL-2, IL-5, IL-6 and gamma interferon (14, 17, 18, 25, 34, 35, 36, 39). IL-5 and IL-6 which serve to enhance IgE synthesis are secreted by TH-2 cells and IL-2 and gamma interferon are secreted by TH-1 cells. (19) Gamma interferon not only promotes switching to IgG but it also antagonizes switching to IgE. (14, 18, 39)

Recent studies have indicated that severe allergic diseases such as atopic dermatitis are associated with expansion of TH2 cells. (25) It was also found that most of these allergen specific T-cell clones from these allergic sub-

jects produce high levels of IL-4 and IL-5, with low levels of gamma interferon. (25).

It is possible that a mast cell dependent pathway of IgE formation may also be relevant in the allergen mediated maintenance of local allergic reactions. (45, 48) Allergic reactions mediated within minutes after exposure to allergens usually involve IgE, mast cells, basophils and several other mediators. When two high affinity receptors for IgE are bridged by antigen on mast cells, a number of intracellular events are triggered. (32) The mast cell degranulates releasing numerous modulators (27, 45) which include IL-4. (32, 33, 48) Lymphokines produced as a result of allergen-induced activation of mast cells may lead to production of new IgE either directly or by providing selective T-Cell differentiation towards IL-4 production. (17, 48)

Attempts to measure IL-4 in cord samples using the Quantikine Human IL-4 immunometric "sandwich" enzyme immunoassay proved futile. Readings for the standard curve were very good, however all patient values and controls read less than zero. Information obtained from the manufacture's research department indicate the half-life of IL-4 is approximately 60 minutes and the interleukin clears the human system within 48 hours.

Lymphocytes and monocytes produce many soluble products that appear to control immune reactivity that results in many of the acute and chronic inflammatory changes accompanying immune reactions. (32) In Japan where allergic disease affects 30% of the population, researchers have proposed that cord levels of sCD23 may serve as predictive indicators of future atopic disease. (23) In 1990 Kim et al. (23) reported that significant elevations in cord CD23 levels occurred in 15 infants who later developed persistent atopic symptoms. Their levels were consistently and notably higher than CD23 levels in 53 infants who remained healthy (normal). (23)

In a 1992 report, Kay et al. (19) provided evidence that T cells are involved in the pathogenesis of chronic asthma. He showed that serum levels of sCD25 in acute asthmatics were significantly elevated as compared to normal control groups. Additionally Walker et al. in 1991 (49) reported flow cytometric analysis of T cell activation markers revealed asthmatic individuals are characterized by increased numbers of CD25 bearing CD4+ T cell subsets. Moreover there was a high correlation observed between the degree of airway obstruction, the number of peripheral T lymphocytes expressing CD25 and the concentration of soluble CD25. (49)

HYPOTHESIS: If sCD23 and sCD25 actually play a major role in the initial production of in vivo IgE synthesis, serum levels should be significantly different between allergic and non-allergic individuals. It is hypothesized that elevated cord levels of sCD23 and sCD25 may serve as predictive indicators of future atopy and/or asthma

In the immunology literature the terms FCεRII and CD23 are used for the low affinity receptor for the Fc portion of IgE antibody. (4, 13, 24, 35, 36) The human CD23 molecule is a distinctive immunoglobulin receptor because it is the only known Fc Receptor which does not belong to the same gene superfamily as its ligand. (9, 14, 46). This FC receptor is a structurally unusual receptor in that the carboxyl terminal portion of the CD23 protein resides at the cell exterior and the amino terminus is cytoplasmic. (4, 11, 14)

This 45 kDa type II membrane glycoprotein shares a lectin-homology domain with a large family of calcium dependent (C-type) animal lectins. (2, 3, 4, 6, 8, 9, 13, 14, 20, 46). This homology domain spanning from cysteine 163 through cysteine 282, contains four highly conserved and two partially conserved cysteines, and is completely contained within the 25 kDa sCD23. (8, 9) Furthermore this domain includes the IgE-binding site. (6, 8, 9, 18)

CD23 displays a broad spectrum of biological activities (2, 11) and the role of this molecule may well relate to the cell type on which it is expressed. (4, 8, 9, 18, 26, 27) "In atopic patients CD23 is overexpressed on B cells and on monocytes." (8) Activities affiliated with sCD23 in synergy with or without other cytokines are as a differentiation factor for thymocytes, an accessory promotor of B and T cell growth, regulator of IgE synthesis and an inhibitor of monocyte migration. (8, 9, 24, 26)

In man the CD23 gene is found as a single copy gene located on chromosome 19. (8, 9, 27) This gene encodes two transcripts specifically called CD23a and CD23b which may be activated by 2 different promoters, resulting in different initiation sites and alternative splicing of this single gene. (4, 6, 9, 11, 27, 30, 50, 51) Freshly sequestered peripheral blood monocytes from atopic individuals express these two transcripts. (8, 42) These isoforms differ in their 5' untranslated sequences and in their intracytoplasmic amino-terminus domain. (4, 9, 14) Evidence suggests that T cells, B cells, monocytes, eosinophils and Langerhans' cells express CD23b (6, 36) which is involved in IgE antibody-dependent cellular cytotoxicity and IgE-dependent release of mediators. (3, 7) The role of CD23b in IgE regulation is also enforced by the fact that it is preferen-

tially upregulated on B cells by IL-4. Over-expression of CD23b in atopic subjects is best explained by an imbalance in IL-4 and gamma interferon production. (8) On resting B lymphocytes CD23a is exclusively expressed and plays a role in IgE-dependent antigen presentation (7, 9). When cultured in the presence of IL-4, resting B cells express substantial amount of CD23a and CD23b. (12) The expression of CD23 is lost after immunoglobulin isotype switching and during the differentiation of B cells into immunoglobulin secreting cells. (9, 37, 51)

A serine protease autocatalytic mechanism cleaves the 45kd surface CD23 protein into soluble fragments (1, 4, 6, 7, 8, 9, 11, 13) Utilizing SDS-Page this soluble fragment referred to as soluble CD23 has been previously shown to be analogous to the B cell derived IgE binding factor. (13, 24, 18, 45) Cloning studies have demonstrated that sCD23 formation is derived from the carboxy terminal portion of the CD23 molecule (2, 4, 7) and the initial cleavage site is position 81 in the amino acid sequence. (4, 6) Initially soluble CD23 is released as an unstable 37-kDa or 33-kDa molecule which is subsequently transformed into a more stable 25-kDa fragment that retains the capacity to bind IgE. (4, 8, 9, 13, 20, 26)

C-type lectins are generally defined by their activity in binding the carbohydrate moieties of glycoproteins, found on cells and in the extracellular matrix. CD23 however recognizes a motif in the polypeptide structure of IgE, rather than its attached carbohydrate (2, 7, 14) The site of IgE/CD23 interaction is generally agreed to be the Cε3 domain (30, 46) and sCD23 must be dimeric in order to bind IgE. (14, 30) On CD23 the IgE binding site is located within the lectin binding domain. (2, 8, 9)

The Interleukin-2 receptor/CD25 (IL-2R) is a multicomponent receptor which possesses 2 distinct interleukin-2 (IL-2) binding subunits: the α and the β subunits. (29, 40, 51) The α-subunit is also known as the p55 or Tac chain and the β chain is also known as the p70/75 subunit. Resting as well as mature activated lymphocytes constitutively express the β-chain of CD25; the α-chain or Tac molecule is expressed only after mononuclear cell (T,B and M0) activation. (40, 51) The α-subunit exhibits a low affinity binding region, while the β-subunit sustains intermediate binding affinity for IL-2. (29, 40) While the α-subunit is primarily a cytokine binding protein, the β-subunit serves to enhance binding and is also required for signalling. (29, 51) The combined αβ-complex cooperate to yield a high affinity binding site for IL-2. (29, 51)

sCD25 is present at low levels in the sera of all healthy individuals which suggests there is a baseline level of immune activity under normal physiologic conditions. (40) The release of the fully soluble, glycosylated sCD25 protein of approximately 45kDa following mitogen or antigen activation of CD4+ T cells is presumably created by the proteolytic cleavage of cell surface Tac. (40) Binding studies have demonstrated the IL-2 binds to CD25 with affinity similar to that measured for the cell surface Tac molecule. (40)

Histology studies of asthmatic bronchial biopsies and bronchoalveolar lavage have demonstrated the presence of numerous CD25-expressing cells during acute stages of asthma. (5) Additionally CD25+HLA-DR+ T Cells are detectable in the peripheral blood of acute severe asthmatics. (5) Whether sCD25 by virtue of its ability to bind IL-2 plays a role in the regulation of immune response remains to be determined.

Serum sIL-2R studies have suggested that sIL-2R measurements may offer a rapid, reliable and non-invasive indication of activity, response to therapy and prognosis in conditions associated with T & B cell activation.

MATERIALS AND METHODS:

Population: At the University of Arizona within the Respiratory Science Center there is a Specialized Center of Research (SCOR) program that has several ongoing projects pertaining to airway obstructive diseases. One project entitled the Tucson Children's Respiratory Study (CRS) presented the opportunity to determine if soluble levels of CD23 and CD25 are indeed predictive indicators of atopy.

A major goal of the CRS study is to provide a long-term prospective investigation of potential risk factors and their interrelationship in the development of chronic obstructive airway diseases including asthma. (47)

The basic CRS study and all of its components were approved by the Human Subjects Committee of the University of Arizona Health Sciences Center. (47).

The parents of healthy neonates born between May 1980 and October 1984 enrolled in Tucson's largest Health Maintenance Organization (HMO) program at that time were contacted regarding voluntary participation in the CRS program. (47)

It is important to emphasize that the 1,246 participants enrolled in this longitudinal study were healthy, because most previous studies were hindered by factors such as they

were retrospective in nature and consequently the impact of prior illnesses within the study population could not be adequately evaluated. (47) Additionally selecting participants from this HMO yielded a large representative population of the Tucson community. (47)

During the first ten year period 407 of the 1246 families enrolled were lost to follow-up, leaving a total of 839 index subjects and their families enrolled in the CRS. .

SAMPLES: At the time of birth, cord blood samples were collected these from newborns by the hospital staff. After their use and prior to the disposal of the cord blood, CRS staff collected these samples from them. Serum was separated, aliquoted and frozen. Prist IgE values were measured on aliquots taken from these cord blood samples. Additional aliquots were frozen at -70 degrees centigrade for future immunological studies. Between ages 4 and 9 Respiratory Health In-Depth-1 Evaluations (In-Depth-1) were performed and at the same time serum samples were collected, separated and frozen. Prist IgE values were also measured on aliquots of serum collected during the In-Depth-1 Evaluations.

CD25 Serum samples can be easily frozen, stored indefinitely and thawed as needed without loss of activity. (40)

METHODS: To measure the serum levels of sCD23 and sCD25 Enzyme-linked Immunosorbent Assays (ELISA) were utilized for test studies. ELISA assays provide an accurate, sensitive and relatively inexpensive method for measuring unknown antigens or antibodies in sera. Labelled reagents are stable, easily stored for long periods of time without loss of activity and maximum binding can be reached quite quickly. Commercial ELISA kits readily available from The Binding Site and AMAC Inc. were purchased to measure CD23 and CD25 respectively. Both assays were used according to the manufacturer's instructions.

sCD23 PROTOCOL: The principle of the BidazymeTM soluble CD23 Enzyme Immunoassay (EIA) Kit MK112 is based on a sandwich enzyme immunoassay in which microtitre wells are coated with anti-sCD23 monoclonal antibody. The first step in performing this analysis was to dilute the kit calibrator (sCD23). The 50 µg/L calibrator provides the highest point for the standard curve. The remaining points on the standard curve were derived by making double dilutions beginning with 250-µl of 50 µg/ml into 250 µl of "wash solution" supplied by the manufacture. One hundred microliters of test samples, controls and standards were applied to the appropriately designated well in each assay tray. The tray was covered then incubated in a moist 37 degree C. incubator

for two hours. If sCD23 is present it binds to the mAb attached to the well. After thoroughly washing the wells 3 times with a protein and detergent containing wash buffer the plates were blotted onto absorbent paper. One-hundred microliters of sheep anti-human sCD23, diluted 1:200, was then added to each well. The plates were covered again and incubated for 2 hours so that the second antibody may bind to the sCD23 in the test samples wells. A second wash regime following this incubation period removes any unbound, excess antibody. The plates were blotted dry again, then 100 μ l of horseradish peroxidase (HRP) - conjugated anti-sheep IgG diluted per manufacture's instructions was added to each test well. Horseradish peroxidase catalyzes an enzymatic reaction with a very high turn-over rate, offering good sensitivity with short reaction times. The comparatively small molecular size of HRP allows for excellent removal of excess conjugate and results in relatively lower background readings. After the third 2 hour incubation in which the anti-sheep IgG conjugate binds to sheep anti-human sCD23 present in the wells, the plates were washed for a final time. After blotting 100 μ l of the color reagent substrate, 3,3',5,5'-tetramethylbenzidine (TMB) which reacts with the peroxidase conjugate was added to each well. This substrate allows for extremely sensitive detection of peroxidase labeled complexes. A blue colored reaction

product results within 30 minutes where the conjugate had bound to the plate. After thirty minutes the addition of 100 μ l of a stopping reagent (phosphoric acid) to each well turned the samples bright yellow. The absorbance of the samples on the developed plate was read spectrophotometrically on an ELISA reader programmed at a wavelength of 450nm. The developed color is stable for 30 minutes and the amount of color produced in each sample will be proportional to the concentration of sCD23. In order to establish a calibration curve the optical densities of each standard were plotted on semilog graph paper. Results were calculated from comparison with the calibration curves derived with each new run.

sCD23 CONTROLS: Two different controls were run with each assay. Often controls were included on each tray of samples contained in a test run. The "known" positive control came with the Assay test kit packaged as 1.4 ml of stabilized human sera ready for use. Throughout this study the batch number of the kit control was E452HF. The assigned mean and standard deviation (SD)) were $9.72 \pm 0.85 \mu\text{g/L}$. Statistical analysis of the kit control values achieved from the eight sCD23 assays yielded a mean value of $9.54 \pm 1.34 \mu\text{g/L}$ (SD). The second control run with each assay consisted of an in-house control derived from pooled neonatal cord

samples. The pooled cord blood was aliquoted in one ml vials, then frozen at -20°C . The pooled cord blood yielded a control with a mean value of $4.68 \pm 0.97 \mu\text{g/L}$ (SD).

NORMAL EXPECTED RESULTS FOR sCD23: As recommended by the manufacture the expected normal range is from $1-6 \mu\text{g/L}$.

sCD25 PROTOCOL:

Identification and quantitation of soluble CD25 was made utilizing the Immunotech S.A. (AMAC Inc.) Elisa sandwich technique. This Immunoenzymometric Assay Kit uses 2 distinct noncompetitive monoclonal antibodies (Anti-Tac and 7G7/B6) directed against 2 different epitopes of CD25. Microtitre wells included in the kit are coated by the manufacturer with anti-sIL-2R monoclonal antibody. Initial steps of the assay consist of making two-fold dilutions started by adding $200 \mu\text{l}$ of the 400 pM kit standard into $200 \mu\text{l}$ of diluent. Fifty μl of samples, controls or standards were added to the appropriately designated wells. This step was immediately followed with the addition of a second monoclonal antibody conjugated with alkaline phosphatase to each well. Alkaline phosphatase has a reaction rate that remains linear over a 2 hour period. Consequently the sensitivity of the assay can be improved by allowing the reaction to proceed for a long period of time. The micro-

titre plate was securely covered then rotated at 350 RPM on a microplate rotator 2 hours at room temperature. Following this incubation the wells were washed three times with concentrated wash solution. Two chromogenic substrate tablets, para-nitrophenylphosphate (pNPP), were then dissolved in 30 ml of diethanolamine HCl buffer for each tray of samples run. When dissolved 200 μ l of this substrate was immediately added to each sample well. Following another 30 minute room temperature incubation on the microplate rotator, 50 μ l of NaOH stopping reagent was added to each well. Readings at a wavelength of 405nm should be taken as soon as possible but may be delayed up to 2 hours if the plates are stored in the dark. The intensity of the resulting color was proportional to the sCD25 concentration present in the sample. Patient results were estimated from a standard curve by interpolation.

sCD25 CONTROLS: There were no controls available from the manufacturer. Therefore two distinct in-house controls were run with each assay. Often each control was included on each tray of samples contained in a test run. Statistical analysis was performed on each control value achieved from the seven sCD25 runs. The primary control run with each assay consisted of an in-house control derived from pooled neonatal cord samples. The pooled cord blood was aliquoted

in one ml vials, then frozen at -20. The pooled cord blood yielded a control mean value of 96.36 pM +/- 14.45 pM (SD). The second control was derived from one adult human source. It was frozen serum aliquoted and frozen in one ml vials. The adult sera yielded an control mean value of 45.40 pM +/- 7.84 pM (SD).

NORMAL EXPECTED RESULTS FOR sIL-2R: 70 +/- 45 pM.

The concentration of sIL-2R can be expressed in pg/ml:

1 pM = 42 pg/ml.

Statistical Methods:

When sufficient quantities were available, samples, standards and controls were analyzed in duplicate. All control and subject samples were run undiluted. Values were entered into a computer and data were analyzed using the software packages: Package for Social Sciences (SPSS) and Scientific Information Retrieval Data Base Management Systems.

Normal frequency distributions were performed on cord and In-Depth-1 sCD23 and sCD25 values. From this command I derived the total number (n), mean, standard deviation, standard error and the minimum and maximum values for each of the soluble markers.

In order to discover 'the measure of association' between two variables, correlation coefficients are generally utilized. The pearson product-moment correlation coefficient (r) was used in this study to determine the relative strength of association and the linearity of a relationship between two normally distributed variables. (10) Regression plots were performed for cord sCD23, sCD25 vs In-Depth-1 sCD23, sCD25, and cord and In-Depth-1 values vs cord and In-Depth-1 IgE levels. In regression analysis one is looking for the dependence of one variable directly upon another variable.

Correlation coefficients are dimensionless measurements and are therefore not affected by units of measurement. The correlation coefficient, r value, denotes the degree to which one variable is related to change within another variable. (10) The simplest method of describing a relationship is defined by a straight line. If a positive linear relationship exists, such that both variables are elevated together, then the r value approaches +1. If no linear relationship between two variables exists then there is no association and the r value is zero. If one variable increases as another decreases than a negative linear relationship would result and the r value would be expected to approach -1. (10) R value readings of > 0.80 would be

considered indicative of a strong relationship, those < 0.30 would be considered weakly related.

Oneway analysis was used to test the null hypothesis. This procedure is used when you have one variable that is grouped vs another variable which is continuous. A continuous variable is one which can allow an indefinite number of values between two fixed points. Oneway analysis calculates between-group variance and within group variance. The between-group variance is the variation of the group means around the overall mean and the within group variance is the variability of the scores within each group around its own mean. The ratio of the between group values with the within group values yield the F-ratio (degrees of freedom ratio). A large F-ratio provides evidence against the null hypothesis and a small F-ratio does not. (10) The SPSS computer package automatically calculates the exact p-value for the F-ratio assigned.

Using crosstab one can compare the expected value of a variable with the actual value observed. Observations between variables are usually regarded as independent vs dependent variables. Crosstabs were an effective way to look at relationships between two groups of variables when one variable is discrete, such as SCD25 groups with physi-

cian diagnosed asthma (frequency of a "Yes" response). Crosstab analysis yields a chi square value. Chi square is a statistical method used to determine whether a result of an experiment arose by happenstance or not. (10) If there is no significant difference between the observed and the expected value than the chi square values will be small.

RESULTS:

At the onset of this research project in October 1992 four hundred and sixty four In-Depth-1 samples (out of 839 enrolled participants) had been collected from CRS pediatric subjects. A list of subjects with both cord and In-Depth-1 samples was generated. There was sufficient sample for analysis of 340 cord bloods and 333 In-Depth-1 samples for sCD23. There was also sufficient quantities to perform sCD25 analysis on 304 cord bloods and 326 In-Depth-1 serum samples. Not all patients had companion cord and in-depth-1 samples. Refer to Table 1.

The total number of patients represented by these samples is 434. Individuals with companion cord and In-Depth-1 sCD23 samples totaled 254 those with a match for cord and In-Depth-1 sCD25 totaled 221. Those with matched cord sCD23 and sCD25 In-Depth-1 samples numbered 176.

sCD23 and sCD25 are Detectable in Cord Blood:

One of the first questions posed was whether these factors were measurable in the cord specimens and whether maternal levels of sCD23 cross the placenta and add to the sCD23 levels in the neonate. All samples analyzed within the pilot study were derived from military dependents hospitalized at the USAF Hospital, Davis-Monthan Air Force Base. In

Table 1. Total Number of Samples Evaluated

| AGE (years) | TEST | TOTAL (n) | BOYS (n) | GIRLS (n) |
|----------------|-------|--------------|-------------|--------------|
| BIRTH | SCD23 | 340 | 164 | 176 |
| BIRTH | SCD25 | 304 | 147 | 157 |
| 4-9 | SCD23 | 333 | 170 | 163 |
| 4-9 | SCD25 | 326 | 163 | 163 |

Results represent 8 SCD23 and 7 CD25 assay batches with 2 or 3 kits utilized per batch.

my pilot study 18 newborns and their mothers were tested. During labor routine laboratory samples were taken from the mothers. Excess serum was separated from admission lab work, labeled and frozen by laboratory personnel. At the time of birth, cord blood samples were collected from newborns by the military hospital staff. After their use and prior to cord disposal, nursing staff collected samples from the cords and immediately forwarded them to the laboratory. Lab personnel spun down the cord bloods, the serum was separated and frozen. As illustrated in the scatterplot, figure 2, no correlation was found between the mother and the baby sCD23. The correlation coefficient for mother sCD23 vs baby sCD23 was .053 yielding a p value of .083.

All CRS cord samples tested had detectable levels of each marker. The geometric mean for cord CD23 was 0.597 $\mu\text{g/L}$ and the geometric mean for cord CD25 was 1.940 pM. To determine how the data was dispersed throughout each variable population, frequency distribution analyses were performed. When only one side of a normal distribution curve is extended, the curve is said to be skewed. Frequently normal population distribution patterns can be made more symmetrical with the transformation of measured values into logarithmic scale. As seen in figure 3 data my analysis were enhanced by using logarithmic values.

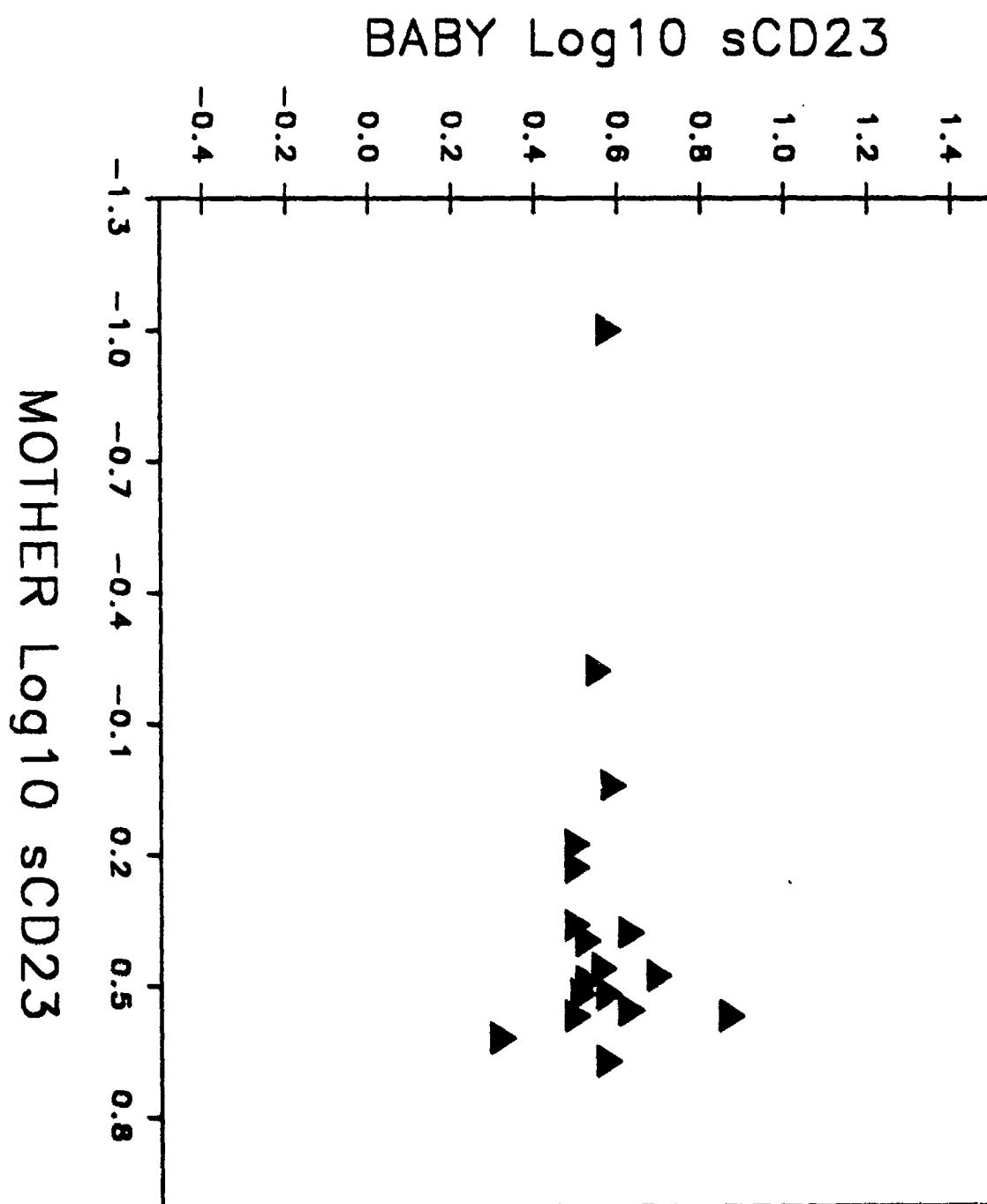


FIGURE 2 Plot of pilot study values for Mother sCD2 values vs corresponding Baby sCD23 values. No significant correlation between mother and baby was found. Correlation coefficient = 0.53. P value equals 0.012.

FIGURE 3. Comparison of frequency distribution of natural number value plots vs Logarithmic conversion plots. Natural numbers depict a skewed normal distribution curve for both sCD23 and sCD25 values. Normal population pattern becomes more symmetrical with Log10 transformation.

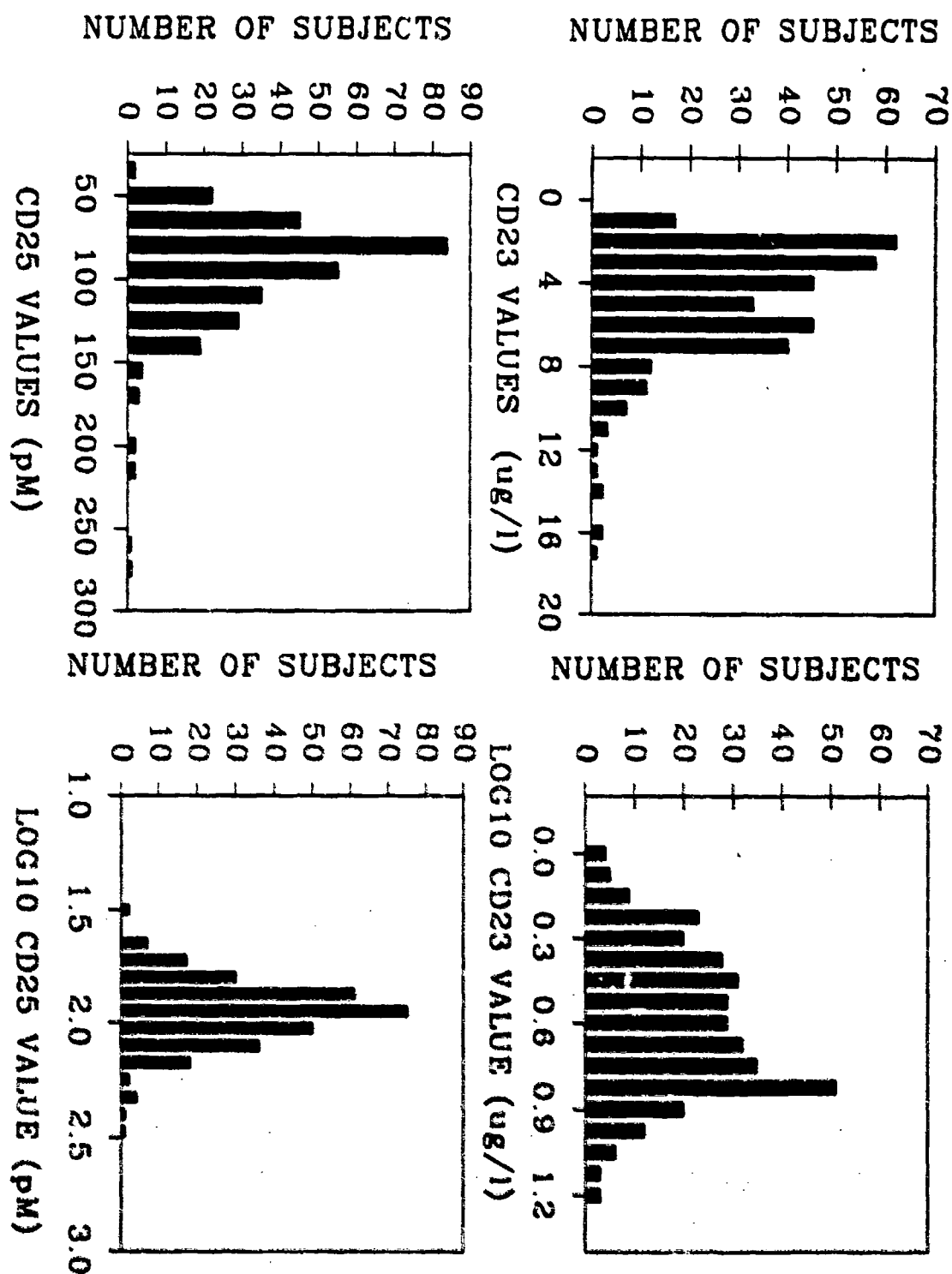


FIGURE 3. Normal Population Distribution Patterns.

FACTORS CHANGE WITH AGE:

The CD25 and CD23 In-Depth-1 levels were found to be significantly different from their respective cord values and therefore represent distinctive populations. The non-paired t-Test (t) and the corresponding probability (p) values for CD23 were $t = 2.773$ and $p < 0.01$ and for CD25 $t = 5.719$ and $p < .001$. Regression plot analysis of individual sCD23 and sCD25 cord values with their corresponding In-Depth-1 values demonstrated correlation. The p value for sCD23 was 0.051 and the p value for sCD25 was .004. The p value from the non-paired t-Test provided in Table 2 illustrate each immunological factor displays a tendency to decrease with age.

The Relationship of sCD23 AND sCD25 levels with Gender:

In this study gender did not prove to have a significant impact on the sCD23 and sCD25 values obtained. As seen in Table 3, Appendix 19 and Appendix 20 the mean cord and In-Depth-1 sCD23 and sCD25 values between the sexes were not significantly different.

TABLE 2. CHANGES IN FACTORS WITH AGE

| <u>AGE</u> | <u>NUMBER</u> | <u>Log10 sCD23</u> <u>MEAN VALUE $\mu\text{g/L}$</u> | <u>SIGNIFICANCE</u> |
|---------------|---------------|--------------------------------------------------------------------|---------------------|
| CORD | 340 | 0.597 +/- .256 | P < 0.01 |
| IN-DEPTH | 333 | 0.530 +/- .291 | |
| <u>FACTOR</u> | <u>NUMBER</u> | <u>Log10 sCD25</u> <u>MEAN VALUE pM</u> | <u>SIGNIFICANCE</u> |
| CORD | 304 | 1.949 +/- .139 | P < 0.001 |
| IN-DEPTH | 326 | 1.853 +/- .2169 | |

The sCD23 and sCD25 In-Depth-1 mean values are significantly decreased from their respective cord values.

TABLE 3. THE RELATIONSHIP OF sCD23 AND sCD25 LEVELS WITH GENDER

| | CD23 $\mu\text{g/L}$ | | CD25 pM | |
|---------------------|----------------------|-------|---------|-------|
| | BOYS | GIRLS | BOYS | GIRLS |
| CORD | | | | |
| N | 164 | 176 | 147 | 157 |
| $\log_{10} \bar{X}$ | .584 | .609 | 1.95 | 1.95 |
| SD | .254 | .258 | .144 | .135 |
| IN-DEPTH | | | | |
| N | 170 | 163 | 163 | 163 |
| $\log_{10} \bar{X}$ | .541 | .519 | 1.85 | 1.85 |
| SD | .311 | .270 | .235 | .196 |

There are no significant differences in marker values based on gender.

Relationship of Soluble Markers with IgE Levels:

The sCD23 and sCD25 levels showed no significant correlation with cord and In-Depth-I IgE levels. The pearson correlation coefficients values for cord sCD23 and sCD25 vs IgE are $-.031$ and -0.011 respectively. For In-Depth-1 vs IgE the pearson coefficient correlation values for sCD23 and sCD25 are $.049$ and $.079$ respectively. Refer to Figures 4 and 5 plus Appendix 2, 3, 4, and 5 for further detail.

The Predictive Nature of Cord Levels for Childhood Asthma, Hayfever and/or eczema:

Neither cord sCD23 or sCD25 appear to be predictive indicators of future asthma or hayfever. Figures 6, 7, and 8 illustrate that there is no significant correlation between cord sCD23 values and the incidence of physician diagnosed asthma, hayfever or eczema at age 6. Reviewing figures 9, 10, and 11 one sees similarly that no significant correlation is detectable between sCD25 physician diagnosed asthma, hayfever and eczema at age 6.

Do sCD23 and sCD25 Levels Correlate with Each Other?

Figure 12 illustrates that there is no significant correlation associated with these two soluble factors. Low levels of sCD25 from Group 1 and high levels of sCD25 yield approximately the same mean sCD23 value.

FIGURE 4. Open bars: Plot of LGIGEKID (kids In-Depth-I IgE values) by levels of LOG10 Cord sCD23 GROUPED by 20%

| Value | Label | Mean | Std Dev | Std Error | Cases |
|-------|-------------|--------|---------|-----------|-------|
| 1.00 | LE.35 | 1.5276 | .7866 | .0983 | 64 |
| 2.00 | GT.35 LE.53 | 1.6016 | .7724 | .0937 | 68 |
| 3.00 | GT.53 LE.69 | 1.6365 | .7930 | .0955 | 69 |
| 4.00 | GT.69 LE.82 | 1.4456 | .6876 | .0810 | 72 |
| 5.00 | GT.82 | 1.5506 | .6467 | .0796 | 66 |

Pearson Coefficient Value: $-.0232$

Solid Bars Plot of LGIGEKID (kids In-Depth-I IgE values by levels of GP5I23 (LOG10 IN-DEPTH sCD23 grouped by 20%).

| Value | Label | Mean | Std Dev | Std Error | Cases |
|-------|-------------|--------|---------|-----------|-------|
| 1.00 | LE.35 | 1.5219 | .6493 | .0831 | 61 |
| 2.00 | GT.35 LE.50 | 1.5936 | .8067 | .0951 | 72 |
| 3.00 | GT.50 LE.60 | 1.4763 | .7355 | .0919 | 64 |
| 4.00 | GT.60 LE.74 | 1.7834 | .7785 | .0951 | 67 |
| 5.00 | GT.74 | 1.6394 | .7781 | .0944 | 68 |

Pearson Coefficient Value: $-.00026$

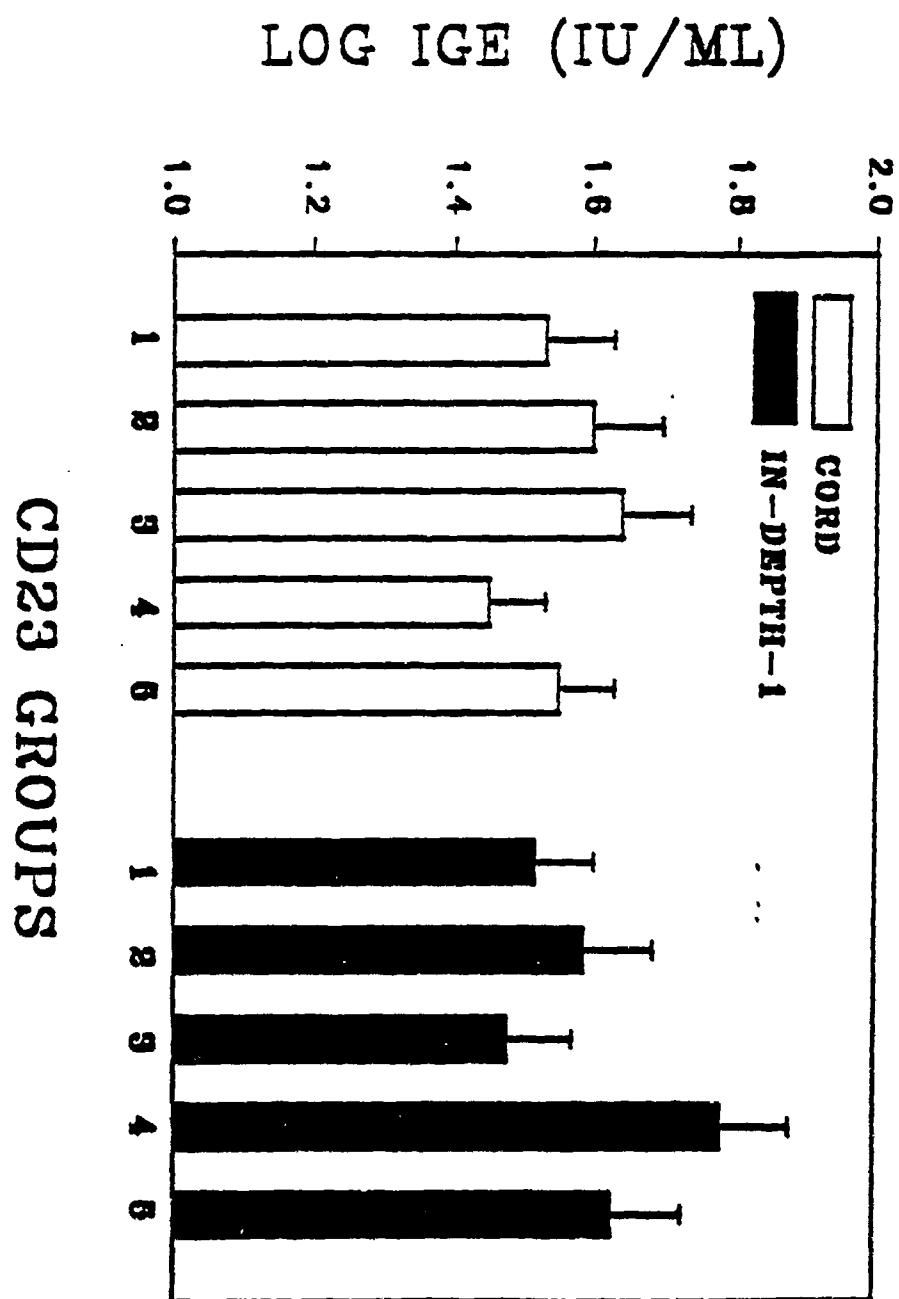


FIGURE 4. sCD23 vs Year 6 Log IgE.

FIGURE 5. Open Bars: Plot of LGIGEKID (kids IDI IgE) by levels of GP5C25 (CORD sCD25 LOG10 grouped by 20%).

| Value | Label | Mean | Std Dev | Std Error | Cases |
|-------|-----------------|--------|---------|-----------|-------|
| 1.00 | LE 1.85 | 1.6391 | .7336 | .0955 | 59 |
| 2.00 | GT 1.85 LE 1.92 | 1.4829 | .7757 | .0985 | 62 |
| 3.00 | GT 1.92 LE 1.98 | 1.5150 | .7007 | .0905 | 60 |
| 4.00 | GT 1.98 LE 2.07 | 1.7370 | .6729 | .0862 | 61 |
| 5.00 | GT 2.071 | 1.5013 | .7474 | .0965 | 60 |

Pearson Coefficient Value: 0.0782

FIGURE 5. Solid Bars: Plot of LGIGEKID (kids IDI IgE) by levels of GP5I25 (LOG10 IN-DEPTH sCD25 grouped by 20%)

| Value | Label | Mean | Std Dev | Std Error | Cases |
|-------|-----------------|--------|---------|-----------|-------|
| 1.00 | LE 1.69 | 1.5153 | .8501 | .1097 | 60 |
| 2.00 | GT 1.69 LE 1.81 | 1.5921 | .8127 | .0986 | 68 |
| 3.00 | GT 1.81 LE 1.91 | 1.4739 | .7044 | .0895 | 62 |
| 4.00 | GT 1.91 LE 2.02 | 1.8192 | .6594 | .0788 | 70 |
| 5.00 | GT 2.02 | 1.6573 | .7447 | .0917 | 66 |

Pearson Coefficient Value: 0.0961

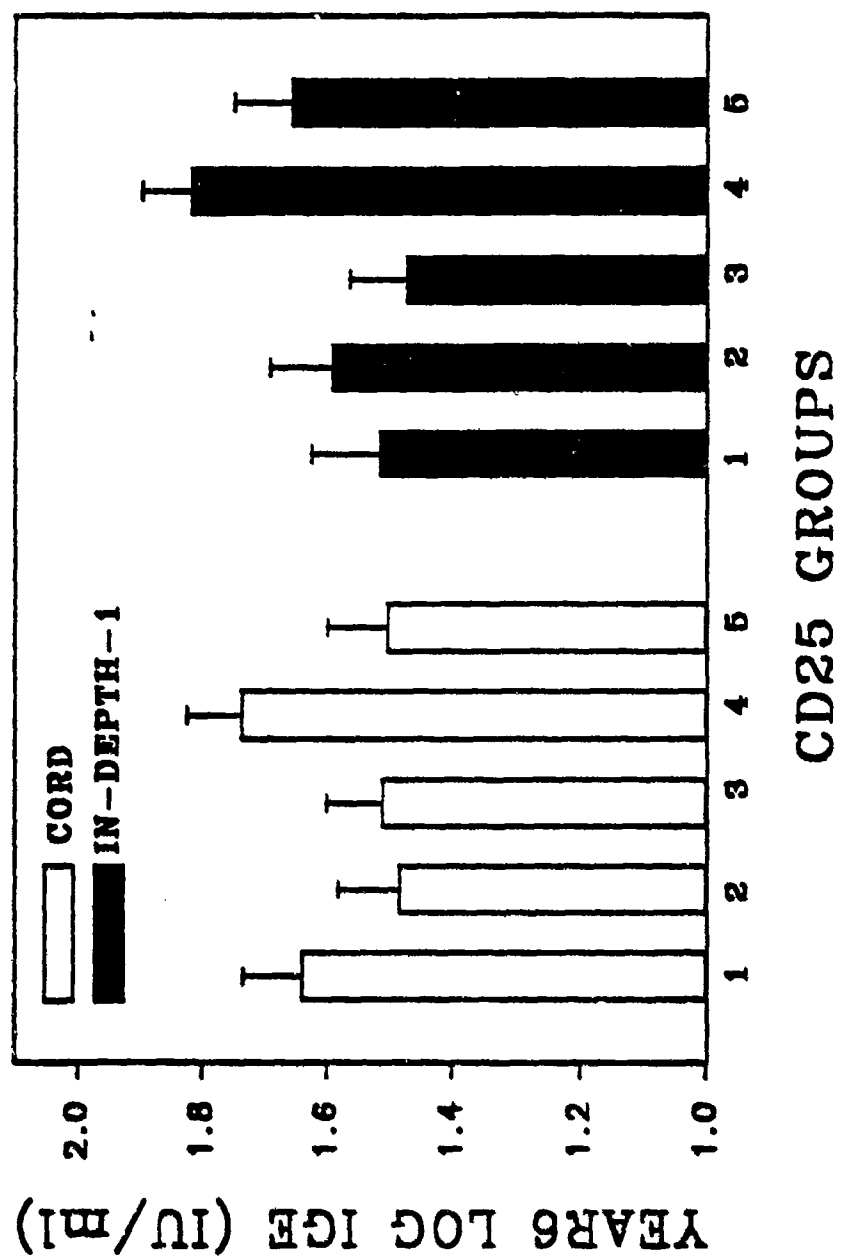


FIGURE 5. SCD25 vs Year 6 Log IGE.

DATA FOR FIGURE 6 CORD CD23 Groups
 Summaries of Y4MDASMA md diagnosed asthma
 By levels of GP5C23 LOG10 CORD CD23 GROUP 20%

| Value | Label | Cases | % Y4MDASMA |
|-------|-------------|-------|------------|
| 1.00 | LE.35 | 64 | 12.5 |
| 2.00 | GT.35 LE.53 | 68 | 23.5 |
| 3.00 | GT.53 LE.69 | 67 | 11.9 |
| 4.00 | GT.69 LE.82 | 71 | 11.3 |
| 5.00 | GT.82 | 67 | 14.9 |

DATA FOR FIGURE 6 In-Depth-I CD23 Group

Summaries of Y4MDASMA md diagnosed asthma
 By levels of GP5I23 LOG10 IN-DEPTH CD23 GROUP 20%

| Value | Label | Cases | % Y4MDASMA |
|-------|-------------|-------|------------|
| 1.00 | LE.35 | 60 | 23.3 |
| 2.00 | GT.35 LE.50 | 72 | 12.5 |
| 3.00 | GT.50 LE.60 | 63 | 7.9 |
| 4.00 | GT.60 LE.74 | 67 | 11.9 |
| 5.00 | GT.74 | 67 | 17.9 |

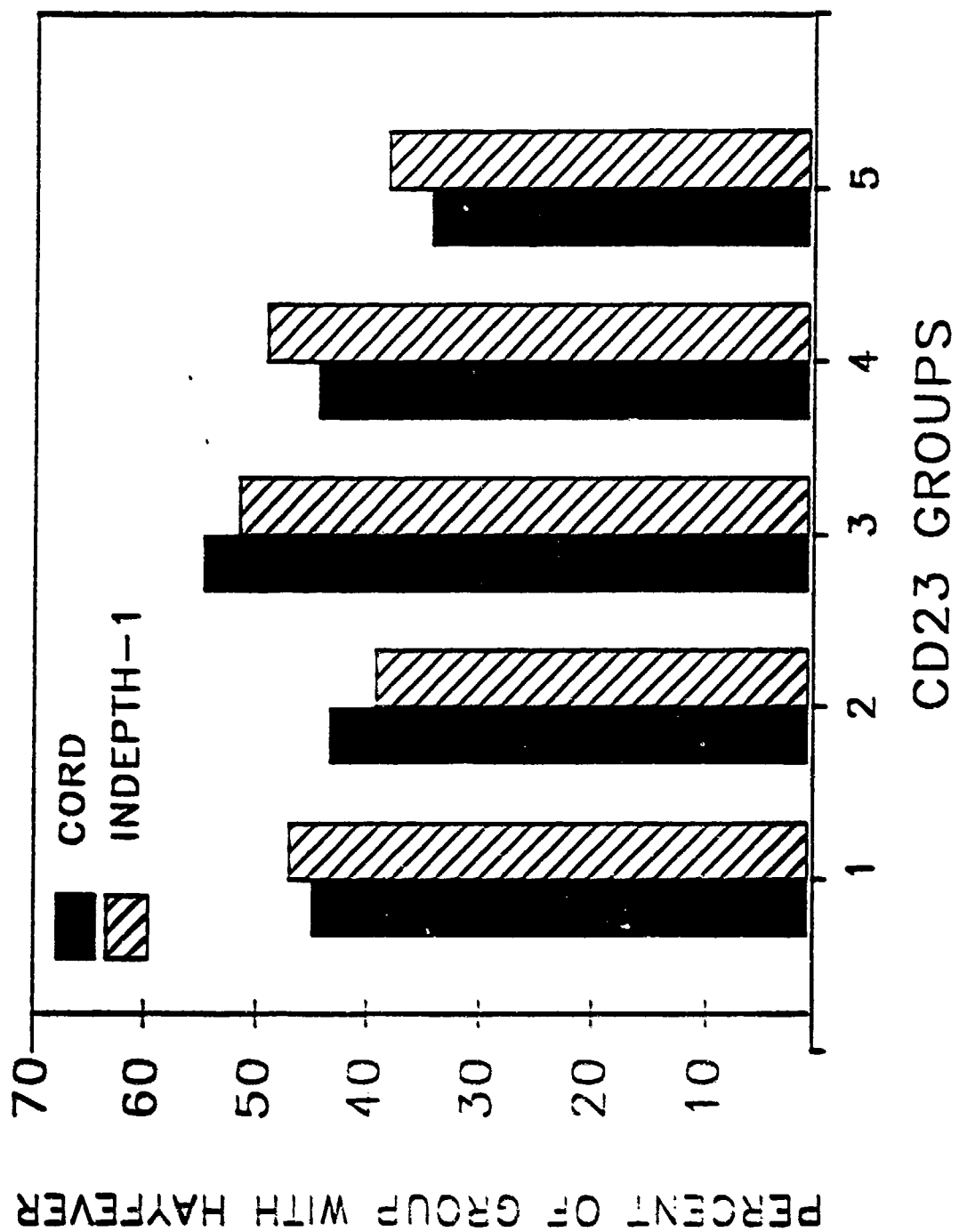


Figure 7. Percent of CD23 Group with Hayfever

FIGURE 7. SUMMARIES OF PHYSICIAN DIAGNOSED HAYFEVER VS levels of LOG10 CORD CD23 grouped by 20% and LOG10 IN-DEPTH CD23 grouped by 20%.

Solid Bars: Cord sCD23

| Value | Label | Cases | % Hayfever |
|-------|-------------|-------|------------|
| 1.00 | LE.35 | 27 | 45.0 |
| 2.00 | GT.35 LE.53 | 27 | 43.5 |
| 3.00 | GT.53 LE.69 | 34 | 54.8 |
| 4.00 | GT.69 LE.82 | 29 | 44.6 |
| 5.00 | GT.82 | 20 | 34.5 |

Open Bars: In-Depth-I sCD23

| Value | Label | Cases | % Hayfever |
|-------|-------------|-------|------------|
| 1.00 | LE.35 | 24 | 47.1 |
| 2.00 | GT.35 LE.50 | 24 | 39.3 |
| 3.00 | GT.50 LE.60 | 31 | 51.7 |
| 4.00 | GT.60 LE.74 | 30 | 49.2 |
| 5.00 | GT.74 | 23 | 38.3 |

No significant correlation is observed between cord or In-Depth-I sCD23 Values and physician diagnosed Hayfever.

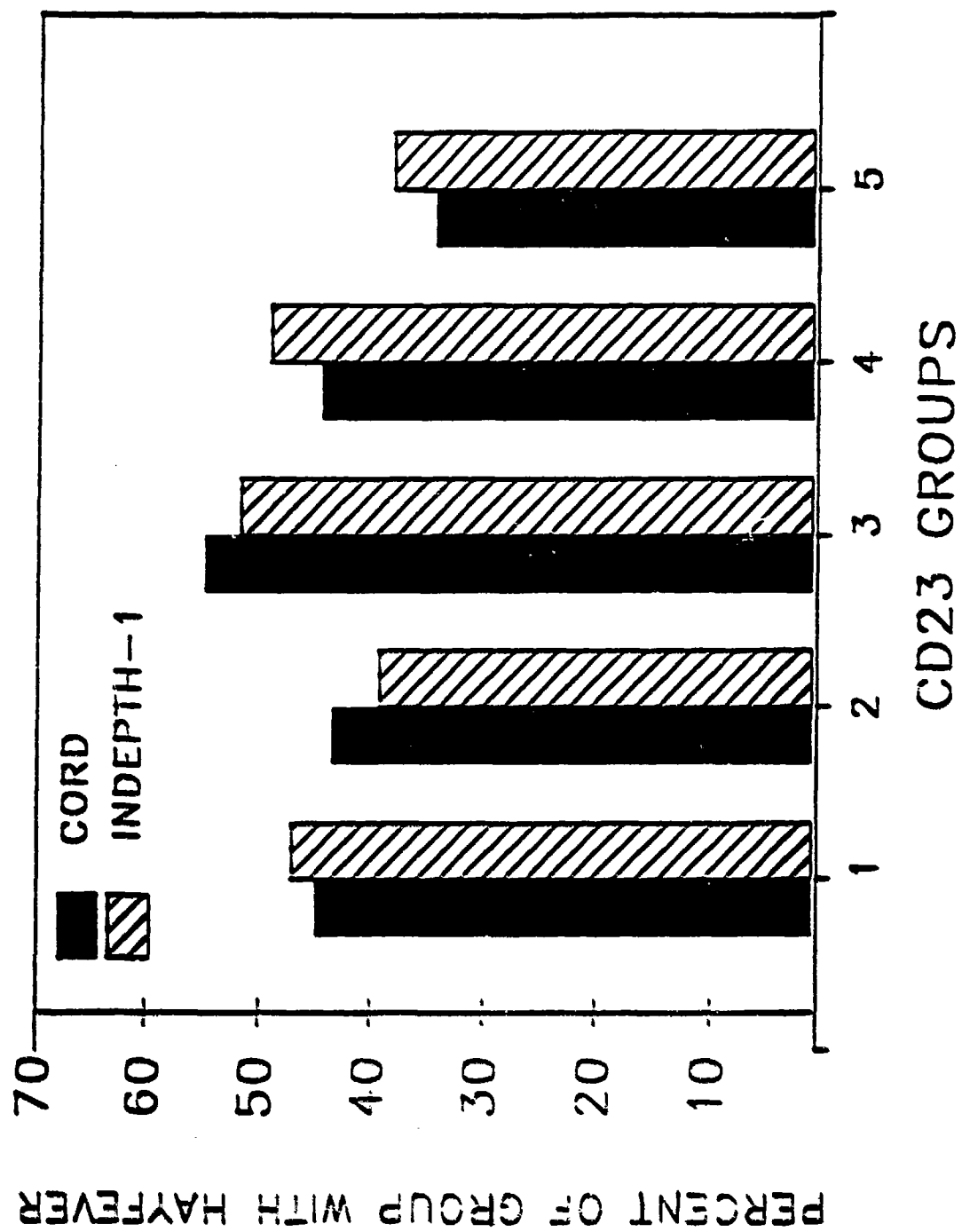


Figure 7. Percent of CD23 Group with Hayfever

FIGURE 8. Summaries of Eczema by levels of LOG10 CORD sCD23 grouped by 20% and LOG10 In-Depth-1 sCD23 grouped by 20%.

Solid Bars: Cord sCD23

| Value | Label | Cases | % Eczema |
|-------|-------------|-------|----------|
| 1.00 | LE.35 | 8 | 12.5 |
| 2.00 | GT.35 LE.53 | 10 | 14.7 |
| 3.00 | GT.53 LE.69 | 10 | 14.5 |
| 4.00 | GT.69 LE.82 | 6 | 8.5 |
| 5.00 | GT.82 | 10 | 15.2 |

Open Bars: In-Depth-1 sCD23

| Value | Label | Cases | % Eczema |
|-------|-------------|-------|----------|
| 1.00 | LE.35 | 11 | 18.3 |
| 2.00 | GT.35 LE.50 | 7 | 9.7 |
| 3.00 | GT.50 LE.60 | 7 | 10.9 |
| 4.00 | GT.60 LE.74 | 10 | 14.9 |
| 5.00 | GT.74 | 8 | 11.9 |

No significant correlation is observed between cord or In-Depth-1 sCD23 values and subjects with a history of eczema.

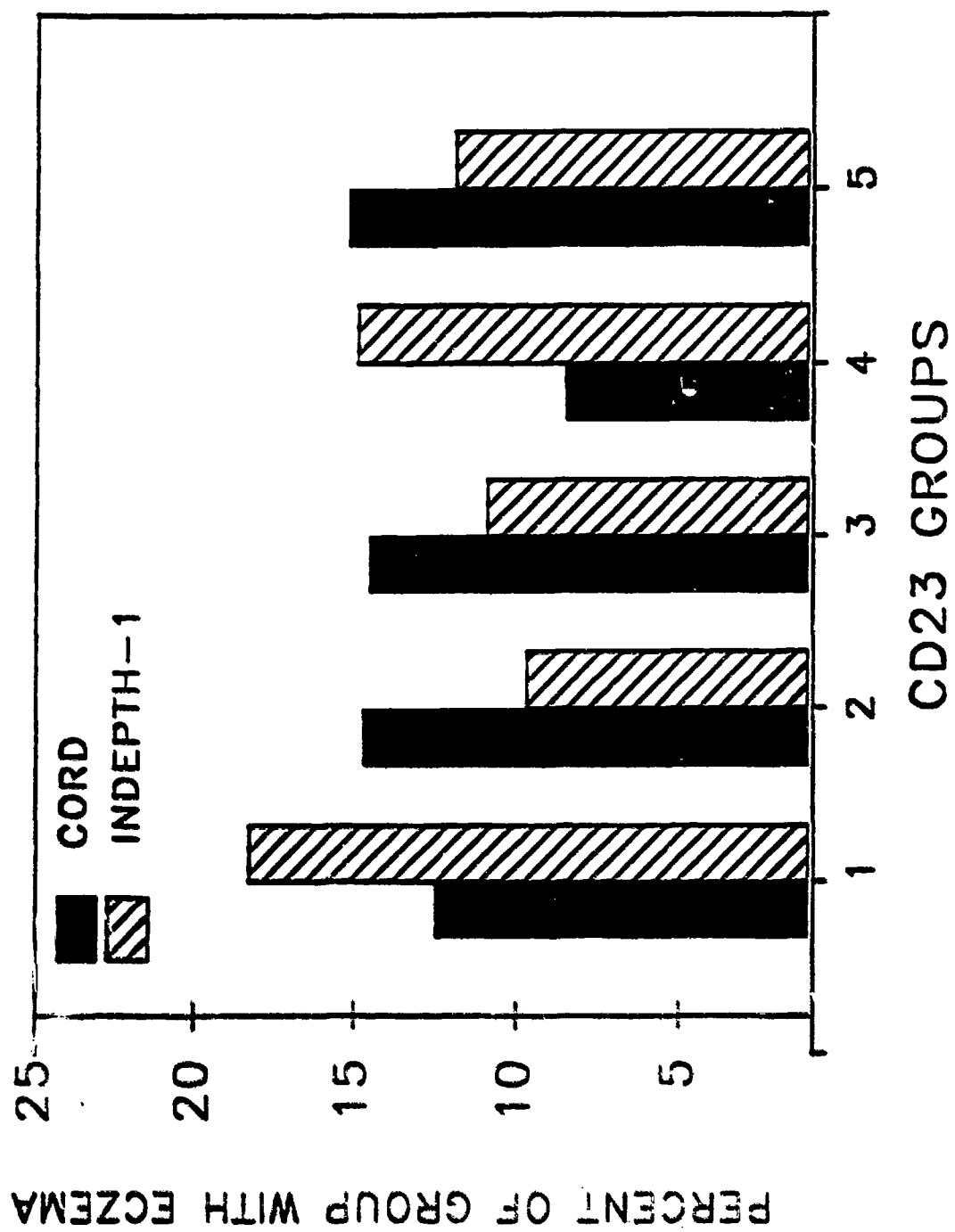


Figure 8. Percent of CD23 Group with Eczema

DATA FOR FIGURE 9 Cord CD25 Group

Summaries of Y4MDASMA md diagnosed asthma

By levels of GP5C25 CORD CD25 LOG10 GROUPED 20%

| Value | Label | Cases | % Y4MDASMA |
|-------|------------------|-------|------------|
| 1.00 | LE 1.846 | 59 | 13.6 |
| 2.00 | GT 1.846 LE1.915 | 62 | 12.9 |
| 3.00 | GT 1.915 LE1.98 | 61 | 11.5 |
| 4.00 | GT 1.98 LE2.071 | 61 | 18.0 |
| 5.00 | GT 2.071 | 59 | 18.6 |

DATA FOR FIGURE 9 In-Depth-I CD25 GROUP

Summaries of Y4MDASMA md diagnosed asthma

By levels of GP5I25 LOG10 IN-DEPTH CD25 GROUP 20%

| Value | Label | Cases | % Y4MDASMA |
|-------|-----------------|-------|------------|
| 1.00 | LE 1.69 | 59 | 15.3 |
| 2.00 | GT 1.69 LE 1.81 | 66 | 16.7 |
| 3.00 | GT 1.81 LE 1.91 | 61 | 9.8 |
| 4.00 | GT 1.91 LE 2.02 | 70 | 21.4 |
| 5.00 | GT 2.02 | 66 | 9.1 |

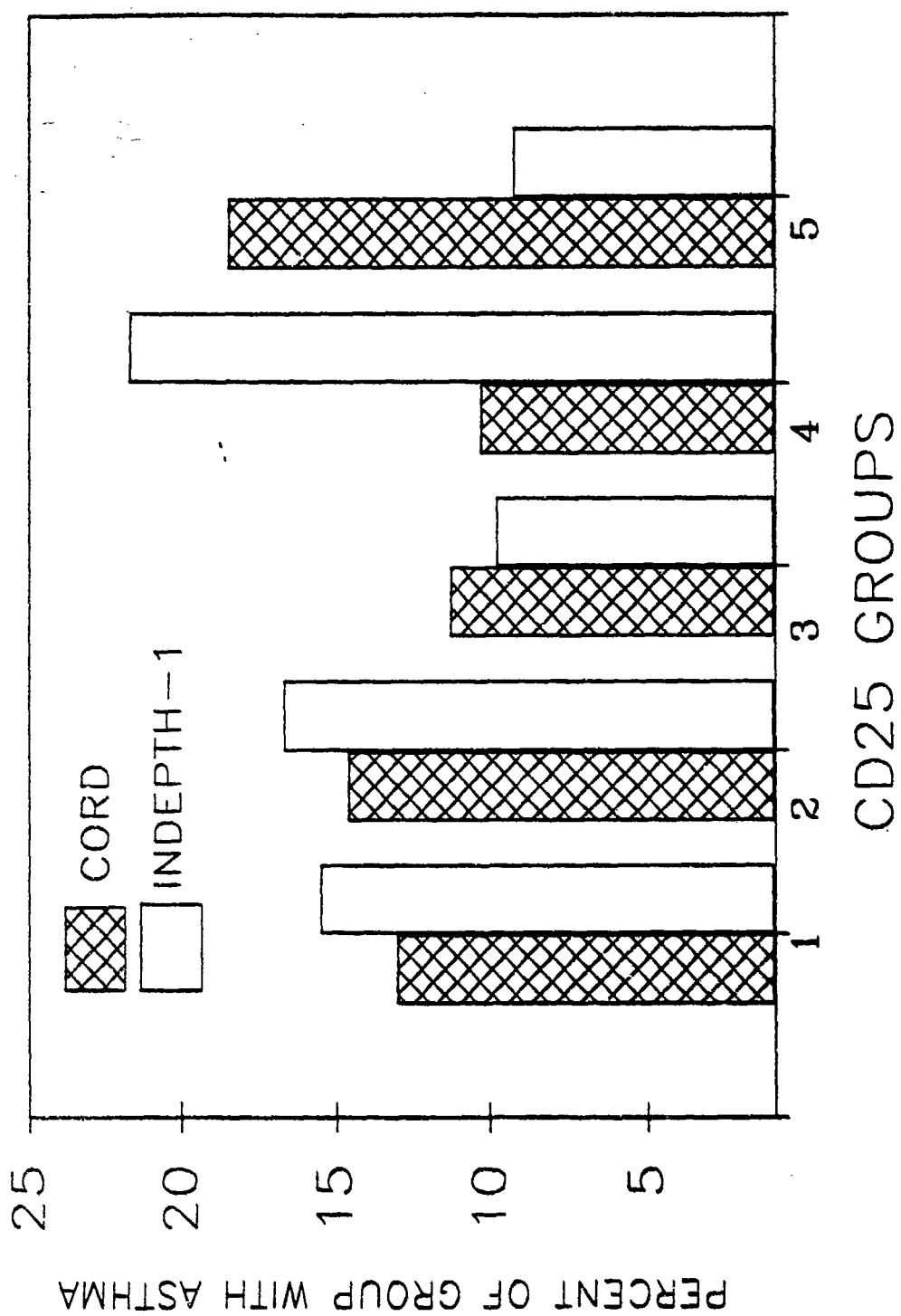


FIGURE 9. Percent of sCD25 Group with Asthma

FIGURE 10. SUMMARIES of PHYSICIAN DIAGNOSED HAYFEVER VS levels of CORD CD25 LOG10 grouped by 20% and LOG10 IN-DEPTH CD25 grouped by 20%.

HATCHED BARS: Cord sCD25

| Value | Label | Cases | % Hayfever |
|-------|-----------------|-------|------------|
| 1.00 | LE 1.85 | 24 | 47.1 |
| 2.00 | GT 1.85 LE 1.92 | 25 | 42.4 |
| 3.00 | GT 1.92 LE 1.98 | 23 | 42.6 |
| 4.00 | GT 1.98 LE 2.07 | 28 | 48.3 |
| 5.00 | GT 2.07 | 22 | 41.5 |

OPEN BARS: In-Depth-1 sCD25

| Value | Label | Cases | % Hayfever |
|-------|-----------------|-------|------------|
| 1.00 | LE 1.69 | 17 | 33.3 |
| 2.00 | GT 1.69 LE 1.81 | 34 | 56.7 |
| 3.00 | GT 1.81 LE 1.91 | 20 | 39.2 |
| 4.00 | GT 1.91 LE 2.02 | 29 | 45.3 |
| 5.00 | GT 2.02 | 29 | 47.5 |

No significant correlation is observed between cord or In-Depth-I sCD25 Values and physician diagnosed Hayfever.

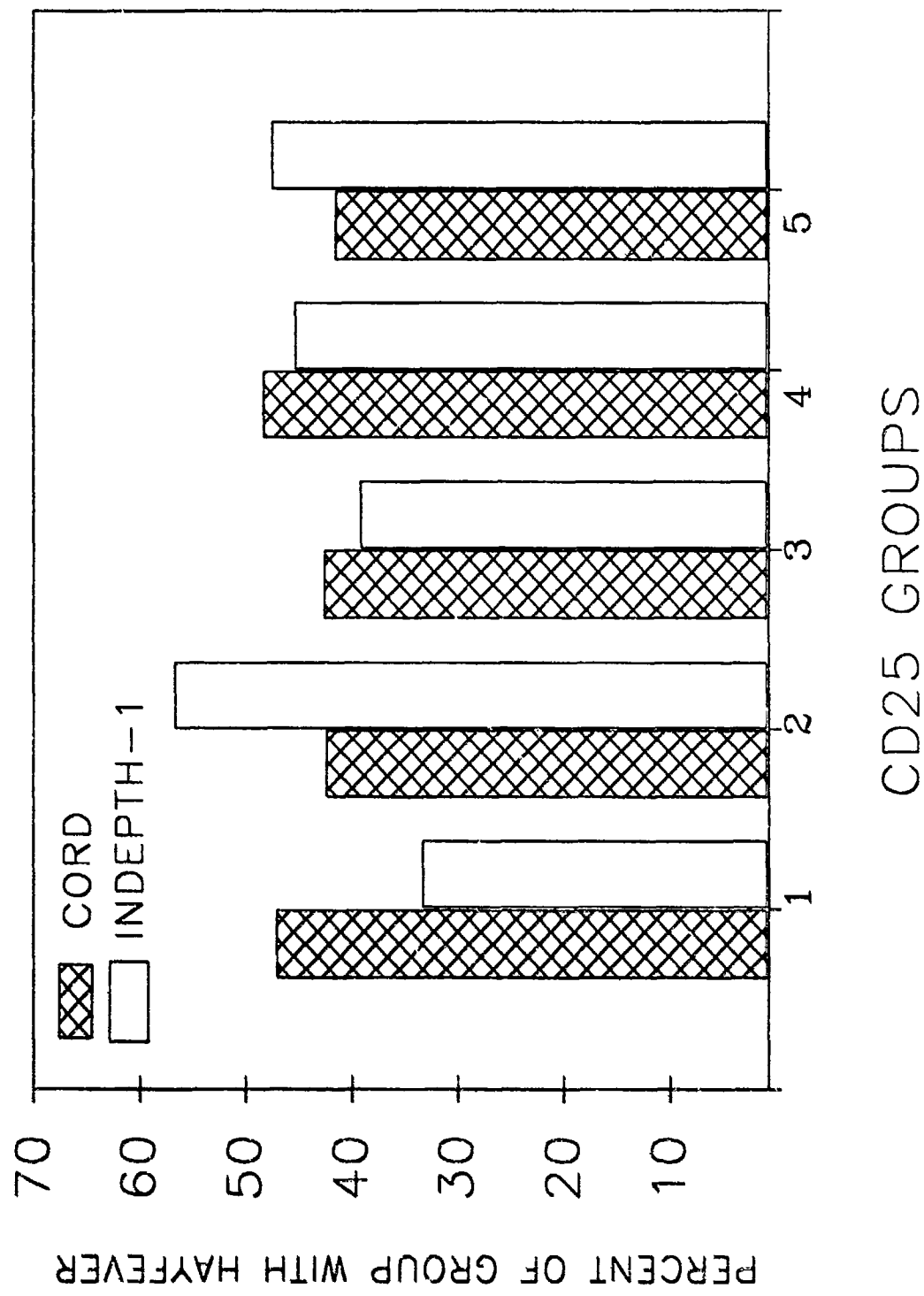


FIGURE 10 Percent of sCD25 Group with Hayfever

FIGURE 11. Summaries of Eczema by levels of Cord CD25 LOG10 grouped by 20% and LOG10 IN-DEPTH CD25 grouped by 20%.

Hatched Bar: Cord sCD25

| Value | Label | Cases | % Eczema |
|-------|------------------|-------|----------|
| 1.00 | LE 1.85 | 8 | 13.6 |
| 2.00 | GT 1.85 LE 1.92 | 9 | 14.8 |
| 3.00 | GT 1.92 LE 1.98 | 9 | 14.8 |
| 4.00 | GT 1.98 LE 2.071 | 11 | 17.7 |
| 5.00 | GT 2.071 | 5 | 8.3 |

Open Bar: In-Depth-1 sCD25

| Value | Label | Cases | % Eczema |
|-------|-----------------|-------|----------|
| 1.00 | LE 1.69 | 5 | 8.5 |
| 2.00 | GT 1.69 LE 1.81 | 9 | 13.4 |
| 3.00 | GT 1.81 LE 1.91 | 8 | 13.1 |
| 4.00 | GT 1.91 LE 2.02 | 7 | 10.0 |
| 5.00 | GT 2.02 | 13 | 19.7 |

No significant correlation is observed between cord or In-Depth-1 sCD25 values and subjects with a history of eczema.

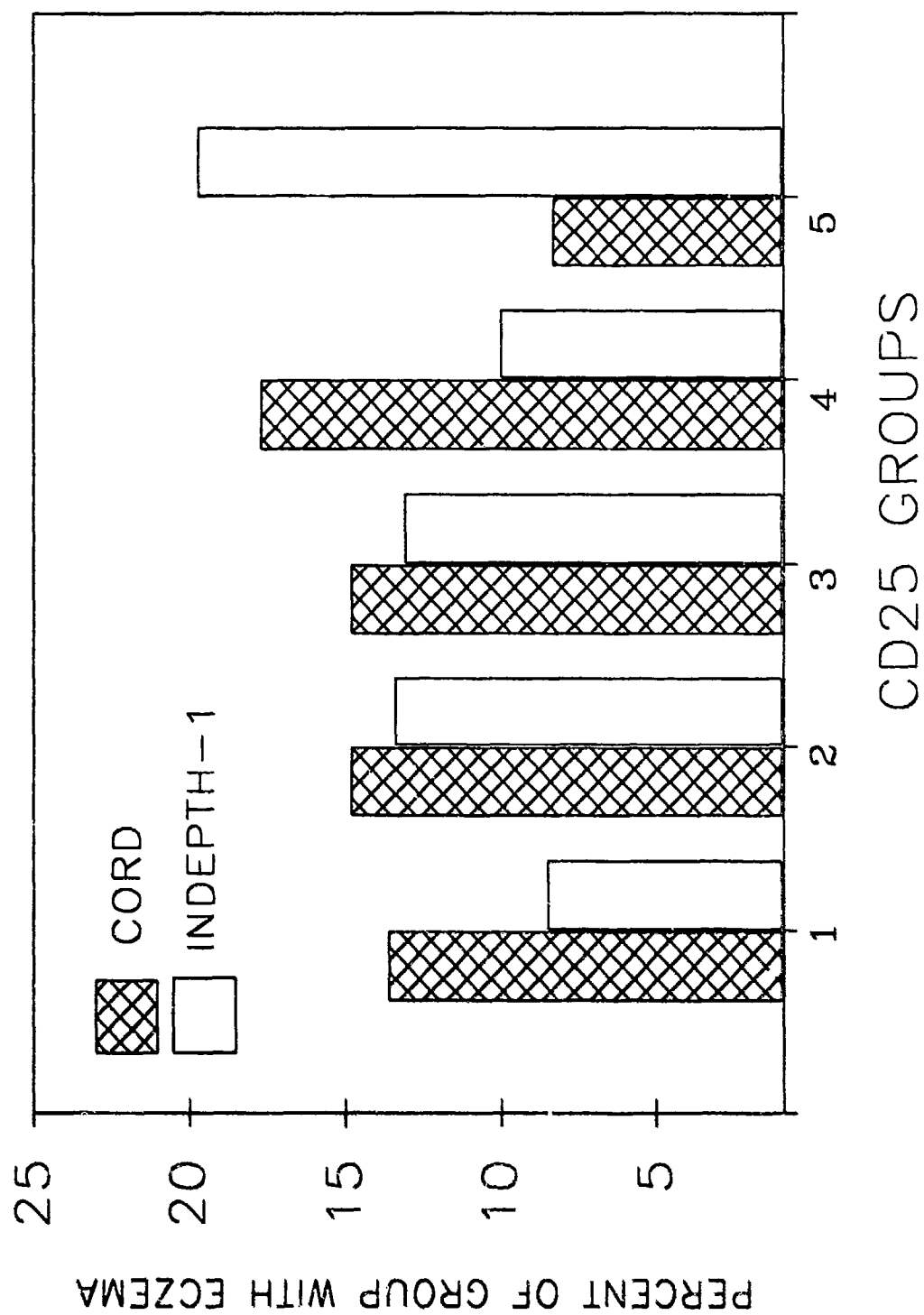


FIGURE 11. Percent of sCD25 Group with Eczema.

FIGURE 12. Summaries of LMIC23 (LOG10 IN-DEPTH MEAN sCD23) by levels of LOG10 IN-DEPTH sCD25 GROUP 20%.

| Value | Label | Mean | Std Dev | Cases |
|-----------------------|-----------------|-------|---------|-------|
| For Entire Population | | .5335 | .2860 | 312 |
| 1.00 | LE 1.69 | .5181 | .3387 | 55 |
| 2.00 | GT 1.69 LE 1.81 | .4982 | .2134 | 67 |
| 3.00 | GT 1.81 LE 1.91 | .4428 | .3847 | 61 |
| 4.00 | GT 1.91 LE 2.02 | .6213 | .2157 | 69 |
| 5.00 | GT 2.02 | .5781 | .2235 | 60 |

There appears to be no significant correlation between these two soluble immunological factors. As noted in Group 1 and Group 5 the mean CD23 values are very similar.

IN-DEPTH 1 VALUES

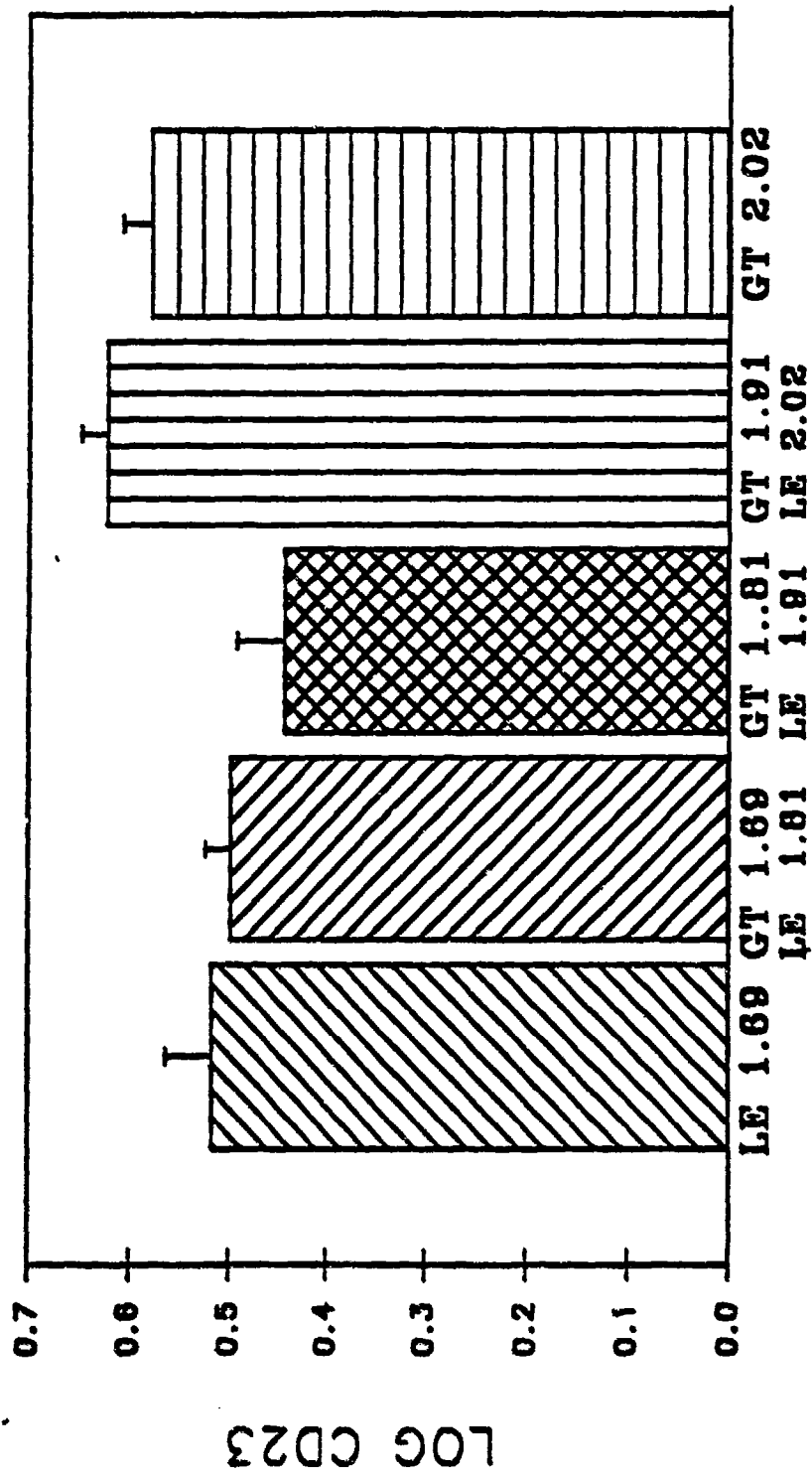


Figure 12. Correlation of In-Depth-1 CD25 Groups with In-Depth-1 CD23 Values

DISCUSSION:

My hypothesis proposes that if sCD23 and sCD25 have a crucial role in the initial production of IgE synthesis, one would anticipate seeing substantial differences in cord serum levels of both markers between atopic and non-atopic subjects. Previous findings of Japanese researchers actively investigating childhood atopy have demonstrated there are differences in sCD23 and sCD25 levels among allergic and non-allergic age-matched subjects (28, 50). Literature searches however did not reveal any large, longitudinal studies involving cord blood analysis of sCD23 or sCD25 and their relationship with the expression of atopic disease. Although enhanced levels of sCD23 levels associated with IgE have been noted among atopic groups in some small studies, analysis of individual associations have not proven to be significant. This study was conducted to determine if cord sCD23 and/or sCD25 derived from a "healthy population" could serve as predictive indicators of future atopic disease.

Maternal/Cord Study:

Similar to results from the Kim et al. (23) study, this study demonstrated that infant cord sCD23 values do not correlate with their corresponding mothers' serum CD23

levels. These data imply that sCD23 originated from infant mononuclear cells and does not cross the placenta.

Age:

In any given individual the levels of sCD23 remain relatively stable over prolonged periods. Age however seems to become an important concern when analyzing sCD23 levels among neonates, young children and adults. Results from this study and those by Gordon et al. (13) illustrate that sCD23 levels are higher in neonates than in older children; further confirmation of a marked age-dependent variation in sCD23 levels within allergic patients as well as non-allergic children is provided in the 1990 Yanagihara et al. study (50) and the 1991 Matsumoto et al. study. (28) Both studies illustrate that allergic and non-allergic children possess significantly higher levels of serum IgE-BF (sCD23) than the corresponding groups of adults. Also in Jyonouchi et al. (18) found younger children had high sCD23 levels that declined with age and that serum IgE levels increased with age. Jyonouchi et al suggest that sCD23 may decline in parallel to rising IgE levels, possibly as a result of negative feedback mechanisms. (18)

The results of my study also showed age related serum sCD25 level changes in otherwise healthy persons. The same find-

ings were noted in two other studies. (28, 40) In Rubin's 1990 review (40) he reiterates that the highest CD25 levels are attained during infancy, with a gradual decline to typically adult levels by the age of 10 years. Additionally, Matsumoto et al. (28) reported age-related decreases in the serum levels of sCD25 for both allergic and non-allergic individuals.

Sex:

No significant differences were noted in sCD23 levels based on gender. These findings agree with a 1990 study by Kim et al. (23) in which total of 76 cord samples from 36 males and 40 females are examined. Results of this study also reinforce the findings by Rubin et al. that no significant sex-related differences in human sCD25 values exist. (40)

Correlation with IgE:

Results of my study indicate there is no significant correlation between sCD23 or sCD25 levels, and IgE levels at birth or at age 6 (In-Depth-I levels). Although his study group was much smaller, Matsumoto's study (28) yielded results which were comparable to mine. Matsumoto et al. (28) determined there is no correlation between the serum level of sCD23 and IgE in children 1-5, 6-15 or adults. They also state that because bronchial asthmatics displaying

elevated IgE levels have soluble IL-2R values within the normal range, a direct relationship between sIL-2R and IgE productivity is very unlikely. (28)

Rousett et al. (39) in a 1991 study, in which 52 patients with elevated IgE levels were compared to 53 age and sex matched healthy individuals, found that as a group the overall mean value of sCD23 was elevated in patients with high IgE. Their findings differ from mine since the overall mean value for sCD23 in my study was not elevated in subjects possessing elevated levels of IgE. Perhaps such a relationship is only seen among older adults. Comparing values within individual patients however, Rousett et al. also observed there was no significant correlation between sCD23 and serum IgE concentrations.

In the 1988 Kim et al study (21) the investigators note an increase in the percentage of CD23+ lymphocytes in young allergic children in whom they presume sensitization is occurring. Additionally in the 1989 Kim et al. study (22) a close positive correlation between the absolute number of CD23 peripheral blood lymphocytes and the serum level of sCD23 is shown to exist. Although the investigators state the serum level of sCD23 in allergic children is significantly higher than levels of non-allergic children in early

childhood, the correlation between serum levels of sCD23 and IgE among the subjects in each of these groups tended to correlate negatively rather than positively.

In the 1988 Kim et al. study (21) no increase in IgE levels was noted in allergic children over the age of three or in non-allergic children over the age of 6 years. Their data suggest allergen sensitization is essentially complete by age 3 in allergic subjects.

Findings in this study are in strong agreement with findings by Kim et al. studies with regard to sCD23 values in older children. In these populations Kim et al. did find there was no correlation between the serum level of CD23 and that of IgE. (21, 23)

The elevation of cord sCD23 in atopic infants suggests that sCD23 upregulation precedes an increase in IgE synthesis. (23). However the fact that no correlation between sCD23 and IgE is found in older children and adults insinuates that sCD23 has little or no effect on ongoing in-vivo IgE synthesis. (23) Kim et al. propose there is a role for sCD23 in the activation or induction phase of IgE production, rather than in the maintenance phase. (23) Possibly in our study IgE levels in the first few weeks of life might

relate to sCD23 at birth. Such samples however were not available.

sCD23 in Allergic vs Non-Allergic Individuals

In my study there was no correlation found between cord or In-Depth-1 levels of sCD23 or sCD25 and patients diagnosed as allergic vs non-allergic subjects. The mean values for both markers in allergic vs non allergic as seen in Tables 4, 5, 6, 7, were impressively similar. In the 1991 Matsumoto et al. study (28), the researchers also failed to find any deviation from normal sCD23 values in children who were diagnosed with atopic eczema or bronchial asthma.

Cord serum levels of CD23 in allergic children however have been experimentally demonstrated by Kim et al. in 1990 (23) to be higher than levels in non-allergic children within the same age group. The incidence of subsequent atopic symptoms in the first year of life was also found to increase as the cord level of sCD23 rose in these allergic children. (23) Despite a great overlap, 257 allergic Japanese subjects in the Yanagihara et al. study (50) also had significantly higher sCD23 levels than 117 non-allergic age-matched controls.

Table 4. Summaries of GP5C25 (CORD CD25 LOG10 GROUPED by 20%) by levels of physician diagnosed a. asthma (mdasma), b. hayfever (MDHAY), and c. Eczema (ECZ).

a. Asthma

| Value | Label | Mean | Std Dev | Sum of Sq | Cases |
|---------------------|----------|--------|---------|-----------|-------|
| 1.00 | mdasma | 3.2000 | 1.4554 | 93.2000 | 45 |
| 2.00 | nomdasma | 3.5000 | .7071 | .5000 | 2 |
| 3.00 | noasma | .9569 | 1.4010 | 498.5255 | 255 |
| ----- | | | | | |
| Within Groups Total | | 2.9967 | 1.4074 | 592.2255 | 302 |

b. Hayfever

| Value | Label | Mean | Std Dev | Sum of Sq | Cases |
|---------------------|-------|--------|---------|-----------|-------|
| .00 | NOHAY | 3.0000 | 1.3849 | 234.0000 | 123 |
| 1.00 | MDHAY | 2.9918 | 1.3995 | 236.9918 | 122 |
| 2.00 | MAYBE | 3.1333 | 1.4559 | 61.4667 | 30 |
| ----- | | | | | |
| Within Groups Total | | 3.0109 | 1.3991 | 532.4585 | 275 |

c. Eczema

| Value | Label | Mean | Std Dev | Sum of Sq | Cases |
|---------------------|-------|--------|---------|-----------|-------|
| 1.00 | Yes | 2.9048 | 1.3217 | 71.6190 | 42 |
| 2.00 | No | 3.0194 | 1.4237 | 520.9031 | 258 |
| 9.00 | Maybe | 3.6667 | 1.5275 | 4.6667 | 3 |
| ----- | | | | | |
| Within Groups Total | | 3.0099 | 1.4109 | 597.1888 | 303 |

TABLE 5. Summaries of GP5I25 (LOG10 IN-DEPTH CD25 GROUP 20%)
by levels of a. MDASMA, b. MDHAY and c. Eczema.

a. Asthma

| Value | Label | Mean | Std Dev | Sum of Sq | Cases |
|---------------------|----------|--------|---------|-----------|-------|
| 1.00 | mdasma | 2.9574 | 1.3666 | 85.9149 | 47 |
| 2.00 | nomdasma | 3.3333 | 2.0817 | 8.6667 | 3 |
| 3.00 | noasma | 3.0699 | 1.4112 | 539.6728 | 272 |
| ----- | | | | | |
| Within Groups Total | | 3.0559 | 1.4101 | 634.2544 | 322 |

b. Hayfever

| Value | Label | Mean | Std Dev | Sum of Sq | Cases |
|---------------------|-------|--------|---------|-----------|-------|
| .00 | NOHAY | 3.0579 | 1.4335 | 246.5950 | 121 |
| 1.00 | MDHAY | 3.1473 | 1.3812 | 244.2016 | 129 |
| 2.00 | MAYBE | 2.9459 | 1.4709 | 77.8919 | 37 |
| ----- | | | | | |
| Within Groups Total | | 3.0836 | 1.4151 | 568.6885 | 287 |

c. Eczema

| Value | Label | Mean | Std Dev | Sum of Sq | Cases |
|---------------------|-------|--------|---------|-----------|-------|
| 1.00 | Yes | 3.3333 | 1.4257 | 83.3333 | 42 |
| 2.00 | No | 3.0144 | 1.4065 | 547.9424 | 278 |
| 9.00 | Maybe | 2.6667 | .5774 | .6667 | 3 |
| ----- | | | | | |
| Within Groups Total | | 3.0526 | 1.4053 | 631.9424 | 323 |

TABLE 6. Summaries of GP5C23 (LOG10 CORD CD23 GROUP 20%) by levels of a. Asthma, b. Hayfever, and c. Eczema.

a. Asthma

| Value | Label | Mean | Std Dev | Sum of Sq | Cases |
|---------------------|----------|--------|---------|-----------|-------|
| 1.00 | mdasma | 2.9200 | 1.3974 | 95.6800 | 50 |
| 2.00 | nomdasma | 1.5000 | .7071 | .5000 | 2 |
| 3.00 | noasma | 3.0561 | 1.4056 | 561.1018 | 285 |
| ----- | | | | | |
| Within Groups Total | | 3.0267 | 1.4028 | 657.2818 | 337 |

b. Hayfever

| Value | Label | Mean | Std Dev | Sum of Sq | Cases |
|---------------------|-------|--------|---------|-----------|-------|
| .00 | NOHAY | 3.0365 | 1.4421 | 282.8175 | 137 |
| 1.00 | MDHAY | 2.9124 | 1.3366 | 242.9489 | 137 |
| 2.00 | MAYBE | 3.1818 | 1.4886 | 70.9091 | 33 |
| ----- | | | | | |
| Within Groups Total | | 2.9967 | 1.4010 | 596.6755 | 307 |

c. Eczema

| Value | Label | Mean | Std Dev | Sum of Sq | Cases |
|---------------------|-------|--------|---------|-----------|-------|
| 1.00 | Yes | 3.0000 | 1.4306 | 88.0000 | 44 |
| 2.00 | No | 3.0206 | 1.4018 | 569.8763 | 291 |
| 9.00 | Maybe | 3.3333 | .5774 | .6667 | 3 |
| ----- | | | | | |
| Within Groups Total | | 3.0207 | 1.4021 | 658.5430 | 338 |

TABLE 7. Summaries of GP5I23 (LOG10 IN-DEPTH CD23 GROUP 20%)
by levels of a. Asthma, b. Hayfever, and c. Eczema.

a. Asthma

| Value | Label | Mean | Std Dev | Sum of Sq | Cases |
|---------------------|----------|--------|---------|-----------|-------|
| 1.00 | mdasma | 2.8958 | 1.6011 | 120.4792 | 48 |
| 2.00 | nomdasma | 4.0000 | 1.7321 | 6.0000 | 3 |
| 3.00 | noasma | 3.0396 | 1.3656 | 516.5647 | 278 |
| ----- | | | | | |
| Within Groups Total | | 3.0274 | 1.4045 | 643.0439 | 329 |

b. Hayfever

| Value | Label | Mean | Std Dev | Sum of Sq | Cases |
|---------------------|-------|--------|---------|-----------|-------|
| .00 | NOHAY | 3.1935 | 1.4066 | 243.3548 | 124 |
| 1.00 | MDHAY | 3.0303 | 1.3588 | 241.8788 | 132 |
| 2.00 | MAYBE | 2.7297 | 1.4269 | 73.2973 | 37 |
| ----- | | | | | |
| Within Groups Total | | 3.0614 | 1.3878 | 558.5309 | 293 |

c. Eczema

| Value | Label | Mean | Std Dev | Sum of Sq | Cases |
|---------------------|-------|--------|---------|-----------|-------|
| 1.00 | Yes | 2.9302 | 1.4864 | 92.7907 | 43 |
| 2.00 | No | 3.0421 | 1.3960 | 553.4947 | 285 |
| 9.00 | Maybe | 3.0000 | .0000 | .0000 | 2 |
| ----- | | | | | |
| Within Groups Total | | 3.0273 | 1.4058 | 646.2854 | 330 |

In the Kim et al. study (23) 37 infants with a family history of atopic disorders were found to have higher cord sCD23 than 38 infants without a family history of atopic disorders; (488 +/- 296 and 405 +/-155 pg/ml respectively) but the difference was not significant.

Asthma:

In contrast to my data, the Matsumoto et al study (28) demonstrated soluble CD23 levels of patients aged 6-10 with bronchial asthma were elevated as compared to non-allergic control groups of the same age. Matsumoto reported the serum levels of sCD25 did not differ between the 20 patients with bronchial asthma and 30 non-allergic control subjects evaluated in his study (28). These findings for the sCD25 marker concur with my observations. The mean levels for sCD25 in 45 cord samples and 47 In-Depth-1 samples from physician diagnosed asthmatics were essentially the same as levels in 255 and 272 non-asthmatic respectively. Reference Tables 4, 5, 6, and 7 provide a summary of my findings.

Eczema:

Levels of sCD23 and sCD25 in this study for children with a history of eczema were remarkably similar to levels in children without a history of eczema. Conflicting with my data, Matsumoto et al. (28) state that sCD25 levels from

patients with atopic eczema and with a history of food allergies are significantly increased compared with sCD25 levels in non-allergic controls of the same age. These researchers however did not find any deviation from normal sCD23 values in children medically diagnosed with atopic eczema within the same study.

sCD23 levels were found to be unexpectedly elevated in four patients with ED (ectodermal dysplasia) in a 1991 study by Jyonoudhi et al. (18) In these patients, EOS numbers were not impressively elevated and sCD23 correlation with serum IgE levels was not significant. (18)

Production of sCD23:

The production of sCD23 can be controlled at 2 levels: the first location is at the cleavage site of CD23 and the second is at the level of cellular expression of CD23.

(6) IL-4 treated cells express more CD23 on their surface and in turn they release more sCD23. Increased levels of IgE may serve as a negative feedback mechanism by inhibiting IL-4 from up-regulating membrane CD23. (6) When IgE binds to the low affinity receptor it inhibits cleavage (6, 8) of the sCD23 and thereby prevents the recruitment of new IgE B cells from their CD23+ precursors (13). A steady state level of IgE is therefore maintained. Such an inhibitory

effect would provide an explanation why individuals with enhanced levels of IgE have decreased levels of sCD23. (13)

CONCLUSION:

Generally speaking serum IgE levels do not appear to correlate with serum CD23 or serum CD25 levels at birth or at age 4-9. Additionally no significant difference in serum CD23 and CD25 between allergic and non-allergic individuals was observed, despite significant difference in serum IgE levels. The data contained in this report indicate that cord sCD23 and sCD25 values will not serve as early indicators for future asthma, hayfever or eczema.

Although the findings of this study are negative, it is important to clarify each of the relationships in a general population study. Reports of others (cited in the discussion) that claim relationships may have included unknown biases in selection of subjects and thus markers correlate with something other than allergy.

There is a need to continue to search for and evaluate the role of other immunological factors such as gamma interferon which may have an association with increased incidence of atopy. The ability to identify those individuals at risk for developing atopic associated diseases early in life could allow for the exclusion of allergens from the diet or the prevention of exposure to allergens in various environments.

APPENDIX 1.

Population Ranges

SCD23 Log10 VALUES ($\mu\text{g/L}$)

| | LOW | MEAN | HIGH | Total (n) |
|------------|--------|------|-------|-----------|
| CORD | .000 | .597 | 1.237 | 340 |
| IN-DEPTH-1 | -1.530 | .530 | 1.230 | 333 |

SCD25 Log10 VALUES (pM)

| | LOW | MEAN | HIGH | Total (n) |
|------------|-------|------|------|-----------|
| CORD | 1.505 | 1.95 | 2.44 | 304 |
| IN-DEPTH-1 | .477 | 1.85 | 2.43 | 326 |

APPENDIX 2. DATA FOR FIGURE 4 CORD CD23

Summaries of LGIGEKID kids IDI IgE
By levels of GP5C23 LOG10 CORD CD23 GROUP 20%

| Value | Label | Mean | Std Dev | Std Error | Cases |
|---------------|-------------|--------|---------|-----------|-------|
| 1.00 | LE.35 | 1.5276 | .7866 | .0983 | 64 |
| 2.00 | GT.35 LE.53 | 1.6016 | .7724 | .0937 | 68 |
| 3.00 | GT.53 LE.69 | 1.6365 | .7930 | .0955 | 69 |
| 4.00 | GT.69 LE.82 | 1.4456 | .6876 | .0810 | 72 |
| 5.00 | GT.82 | 1.5506 | .6467 | .0796 | 66 |
| <hr/> | | | | | |
| Within Groups | | | | | |
| Total | | 1.5517 | .7391 | | 39 |

LE = less than or equal to
GT = greater than

Analysis of Variance

| Source | Sum of Squares | D.F. | Mean Square | F | Sig. |
|---------------------|----------------|---------------------|-------------|-------|-------|
| Between Groups | 1.5133 | 4 | .3783 | .6925 | .5975 |
| Linearity | .0991 | 1 | .0991 | .1814 | .6705 |
| Dev. from Linearity | 1.4142 | 3 | .4714 | .8629 | .4605 |
| R = -.0232 | | R Squared = .0005 | | | |
| Within Groups | 182.4626 | 334 | .5463 | | |
| Eta = .0907 | | Eta Squared = .0082 | | | |

APPENDIX 3. DATA FOR FIGURE 4 IN-DEPTH-1 (IDI) CD23

MEANS/VAR LGIGEKID BY GP5I23/STAT ALL.
 Summaries of LGIGEKID kids IDI IgE
 By levels of GP5I23 LOG10 IN-DEPTH CD23 GROUP 20%

| Value | Label | Mean | Std Dev | Std Error | Cases |
|---------------|-------------|--------|---------|-----------|-------|
| 1.00 | LE.35 | 1.5219 | .6493 | .0831 | 61 |
| 2.00 | GT.35 LE.50 | 1.5936 | .8067 | .0951 | 72 |
| 3.00 | GT.50 LE.60 | 1.4763 | .7355 | .0919 | 64 |
| 4.00 | GT.60 LE.74 | 1.7834 | .7785 | .0951 | 67 |
| 5.00 | GT.74 | 1.6394 | .7781 | .0944 | 68 |
| ----- | | | | | |
| Within Groups | | | | | |
| Total | | 1.6055 | .7545 | | 332 |

Analysis of Variance

| Source | Sum of Squares | D.F. | Mean Square | F | Sig. |
|---------------------|----------------|------|-------------|--------|-------|
| Between Groups | 3.7035 | 4 | .9259 | 1.6265 | .1672 |
| Linearity | 1.1622 | 1 | 1.1622 | 2.0416 | .1540 |
| Dev. from Linearity | 2.5413 | 3 | .8471 | 1.4881 | .2176 |

R = .0782 R Squared = .0061

Within Groups 186.1452 327 .5693

Eta = .1397 Eta Squared = .0195

APPENDIX 4. DATA FOR FIGURE 5 CORD CD25

Summaries of LGIGEKID kids IDI IgE

By levels of GP5C25 CORD CD25 LOG10 GROUPED 20%

| Value | Label | Mean | Std Dev | Std Error | Cases |
|---------------------|----------|--------|---------|-----------|-------|
| 1.00 | LE 1.846 | 1.6391 | .7336 | .0955 | 59 |
| 2.00 | GT 1.846 | | | | |
| | LE 1.915 | 1.4829 | .7757 | .0985 | 62 |
| 3.00 | GT 1.915 | | | | |
| | LE 1.980 | 1.5150 | .7007 | .0905 | 60 |
| 4.00 | GT 1.980 | | | | |
| | LE 2.071 | 1.7370 | .6729 | .0862 | 61 |
| 5.00 | GT 2.071 | 1.5013 | .7474 | .0965 | 60 |
| ----- | | | | | |
| Within Groups Total | | 1.5748 | .7271 | | 302 |

Analysis of Variance

| Source | Sum of Squares | D.F. | Mean Square | F | Sig. |
|---------------------|----------------|------|-------------|--------|-------|
| Between Groups | 2.9107 | 4 | .7277 | 1.3765 | .2420 |
| Linearity | .0011 | 1 | .0011 | .0021 | .9637 |
| Dev. from Linearity | 2.9096 | 3 | .9699 | 1.8347 | .1409 |

R = -.0026 R Squared = .0000

Within Groups 157.0069 297 .5286

Eta = .1349 Eta Squared = .0182

APPENDIX 5. DATA FOR FIGURE 5 IN-DEPTH-1 CD25

Summaries of LGIGEKID kids IDI IgE
By levels of GP5I25 LOG10 IN-DEPTH CD25 GROUP 20%

| Value | Label | Mean | Std Dev | Std Error | Cases |
|---------------------|---------|--------|---------|-----------|-------|
| 1.00 | LE 1.69 | 1.5153 | .8501 | .1097 | 60 |
| 2.00 | GT 1.69 | | | | |
| | LE 1.81 | 1.5921 | .8127 | .0986 | 68 |
| 3.00 | GT 1.81 | | | | |
| | LE 1.91 | 1.4739 | .7044 | .0895 | 62 |
| 4.00 | GT 1.91 | | | | |
| | LE 2.02 | 1.8192 | .6594 | .0788 | 70 |
| 5.00 | GT 2.02 | 1.6573 | .7447 | .0917 | 66 |
| <hr/> | | | | | |
| Within Groups Total | | 1.6175 | .7554 | | 326 |

Analysis of Variance

| Source | Sum of Squares | D.F. | Mean Square | F | Sig. |
|--------------------------------------|----------------|------|-------------|--------|-------|
| Between Groups | 4.9012 | 4 | 1.2253 | 2.1470 | .0748 |
| Linearity | 1.7360 | 1 | 1.7360 | 3.0418 | .0821 |
| Dev. from Linearity | 3.1652 | 3 | 1.0551 | 1.8487 | .1382 |
| R = .0961 R Squared = .0092 | | | | | |
| Within Groups | 183.1957 | 321 | .5707 | | |
| Eta = .1614 Eta Squared = .0261 | | | | | |

APPENDIX 6. Crosstab Data for Figure 6: % of CD23 Group with Asthma

Y4MDASMA md diagnosed asthma by GP5C23 LOG10 CORD CD23 GROUP 20%

| | | GP5C23 | | | | | Row Total |
|----------|---------|---------|----------|----------|----------|-------|--------------|
| Y4MDASMA | Count | LE.35 | GT.35 LE | GT.53 LE | GT.69 LE | GT.82 | |
| | Exp Val | .53 | .69 | .82 | | | |
| | Row Pct | 1.00 | 2.00 | 3.00 | 4.00 | 5.00 | |
| | | Col Pct | | | | | |
| mdasma | 1.00 | 8 | 16 | 8 | 8 | 10 | 50 |
| | | 9.5 | 10.1 | 9.9 | 10.5 | 9.9 | 14.8% |
| | | 16.0% | 32.0% | 16.0% | 16.0% | 20.0% | |
| | | 12.5% | 23.5% | 11.9% | 11.3% | 14.9% | |
| nomdasma | 2.00 | 1 | 1 | 0 | 0 | 0 | 2 |
| | | .4 | .4 | .4 | .4 | .4 | .6% |
| | | 50.0% | 50.0% | .0% | .0% | .0% | |
| | | 1.6% | 1.5% | .0% | .0% | .0% | |
| noasma | 3.00 | 55 | 51 | 59 | 63 | 57 | 285 |
| | | 54.1 | 57.5 | 56.7 | 60.0 | 56.7 | 84.6% |
| | | 19.3% | 17.9% | 20.7% | 22.1% | 20.0% | |
| | | 85.9% | 75.0% | 88.1% | 88.7% | 85.1% | |
| Column | | 64 | 68 | 67 | 71 | 67 | 337 |
| Total | | 19.0% | 20.2% | 19.9% | 21.1% | 19.9% | 100.0% |

| Chi-Square | Value | DF | Significance |
|--------------------------|---------|----|--------------|
| Pearson | 8.79279 | 8 | .36008 |
| Likelihood Ratio | 9.02676 | 8 | .34004 |
| Mantel-Haenszel test for | .55799 | 1 | .45507 |

APPENDIX 7. Crosstab Data for Figure 6: % of IDI CD23 Group with Asthma
 Y4MDASMA md diagnosed asthma by GP5I23 LOG10 IN-DEPTH CD23 GROUP 20%

| | Count Exp Val Row Pct Col Pct | GP5I23 | | | | | Row Total |
|--------------|----------------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|---------------|
| | | LE.35 | GT.35 LE | GT.50 LE | GT.60 LE | GT.74 | |
| | | .50 | .60 | .74 | | | |
| | | 1.00 | 2.00 | 3.00 | 4.00 | 5.00 | |
| Y4MDASMA | | | | | | | |
| mdasma | 1.00 | 14 3.8 29.2% 23.3% | 9 10.5 18.8% 12.5% | 5 9.2 10.4% 7.9% | 8 9.8 16.7% 11.9% | 12 9.8 25.0% 17.9% | 48 14.6% |
| nomdasma | 2.00 | 0 .5 .0% .0% | 1 .7 33.3% 1.4% | 0 .6 .0% .0% | 0 .6 .0% .0% | 2 .6 66.7% 3.0% | 3 .9% |
| noasma | 3.00 | 46 50.7 16.5% 76.7% | 62 60.8 22.3% 86.1% | 58 53.2 20.9% 92.1% | 59 56.6 21.2% 88.1% | 53 56.6 19.1% 79.1% | 278 84.5% |
| Column Total | | 60 18.2% | 72 21.9% | 63 19.1% | 67 20.4% | 67 20.4% | 329 100.0% |

| Chi-Square | Value | DF | Significance |
|--------------------------|----------|----|--------------|
| Pearson | 12.38579 | 8 | .13480 |
| Likelihood Ratio | 12.79867 | 8 | .11897 |
| Mantel-Haenszel test for | .28932 | 1 | .59066 |

APPENDIX 8. Crosstab Data for Figure 7: % of Cord CD23 Group with Hayfever

MDHAY MD DIAG HAYFEVER by GP5C23 LOG10 CORD CD23 GROUP 20%

GP5C23

| | Count Exp Val Row Pct Col Pct | LE.35 | GT.35 LE | GT.53 LE | GT.69 LE | GT.82 | Row Total |
|--------------|----------------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|---------------|
| | | 1.00 | .53 2.00 | .69 3.00 | .82 4.00 | 5.00 | |
| MDHAY | .00 | 27 26.8 19.7% 45.0% | 29 27.7 21.2% 46.8% | 22 27.7 16.1% 35.5% | 30 29.0 21.9% 46.2% | 29 25.9 21.2% 50.0% | 137 44.6% |
| MDHAY | 1.00 | 27 26.8 19.7% 45.0% | 27 27.7 19.7% 43.5% | 34 27.7 24.8% 54.8% | 29 29.0 21.2% 44.6% | 20 25.9 14.6% 34.5% | 137 44.6% |
| MAYBE | 2.00 | 6 6.4 18.2% 10.0% | 6 6.7 18.2% 9.7% | 6 6.7 18.2% 9.7% | 6 7.0 18.2% 9.2% | 9 6.2 27.3% 15.5% | 33 10.7% |
| Column Total | | 60 19.5% | 62 20.2% | 62 20.2% | 65 21.2% | 58 18.9% | 307 100.0% |

| Chi-Square | Value | DF | Significance |
|--------------------------|---------|----|--------------|
| Pearson | 5.97079 | 8 | .65050 |
| Mantel-Haenszel test for | .00166 | 1 | .96753 |

APPENDIX 9. Crosstab Data for Figure 7: % of IDI CD23 Group with Hayfever

MDHAY MD DIAG HAYFEVER by GP5I23 LOG10 IN-DEPTH CD23 GROUP 20%

GP5I23

| | Count Exp Val Row Pct Col Pct | LE.35 | GT.35 LE | GT.50 LE | GT.60 LE | GT.74 | Row Total |
|--------|----------------------------------------|-------|----------|----------|----------|-------|--------------|
| | | 1.00 | .50 2.00 | .60 3.00 | .74 4.00 | 5.00 | |
| MDHAY | .00 | 17 | 29 | 23 | 23 | 32 | 124 |
| NOHAY | | 21.6 | 25.8 | 25.4 | 25.8 | 25.4 | 42.3% |
| | | 13.7% | 23.4% | 18.5% | 18.5% | 25.8% | |
| | | 33.3% | 47.5% | 38.3% | 37.7% | 53.3% | |
| MDHAY | 1.00 | 24 | 24 | 31 | 30 | 23 | 132 |
| | | 23.0 | 27.5 | 27.0 | 27.5 | 27.0 | 45.1% |
| | | 18.2% | 18.2% | 23.5% | 22.7% | 17.4% | |
| | | 47.1% | 39.3% | 51.7% | 49.2% | 38.3% | |
| MAYBE | 2.00 | 10 | 8 | 6 | 8 | 5 | 37 |
| | | 6.4 | 7.7 | 7.6 | 7.7 | 7.6 | 12.6% |
| | | 27.0% | 21.6% | 16.2% | 21.6% | 13.5% | |
| | | 19.6% | 13.1% | 10.0% | 13.1% | 8.3% | |
| Column | | 51 | 61 | 60 | 61 | 60 | 293 |
| Total | | 17.4% | 20.8% | 20.5% | 20.8% | 20.5% | 100.0% |

| Chi-Square | Value | DF | Significance |
|--------------------------|---------|----|--------------|
| Pearson | 8.71440 | 8 | .36696 |
| Likelihood Ratio | 8.54081 | 8 | .38250 |
| Mantel-Haenszel test for | 3.14018 | 1 | .07639 |

APPENDIX 10. Crosstab for Figure 8: % of Cord CD23 Group with Eczema

Eczema (Y4C26A1) by GP5C23 LOG10 CORD CD23 GROUP 20%

GP5C23

| Y4C26A1 | Count | GP5C23 | | | | | Row Total |
|--------------|---------|--------|-------|----------|----------|----------|-----------|
| | Exp Val | LE.35 | GT.35 | LE GT.53 | LE GT.69 | LE GT.82 | |
| | Row Pct | | | | | | |
| | Col Pct | 1.00 | 2.00 | 3.00 | 4.00 | 5.00 | |
| 1.00 | 8 | 10 | 10 | 6 | 10 | 44 | |
| | 8.3 | 8.9 | 9.0 | 9.2 | 8.6 | 13.0% | |
| | 18.2% | 22.7% | 22.7% | 13.6% | 22.7% | | |
| | 12.5% | 14.7% | 14.5% | 8.5% | 15.2% | | |
| 2.00 | 56 | 58 | 57 | 64 | 56 | 291 | |
| | 55.1 | 58.5 | 59.4 | 61.1 | 56.8 | 86.1% | |
| | 19.2% | 19.9% | 19.6% | 22.0% | 19.2% | | |
| | 87.5% | 85.3% | 82.6% | 90.1% | 84.8% | | |
| 9.00 | 0 | 0 | 2 | 1 | 0 | 3 | |
| | .6 | .6 | .6 | .6 | .6 | .9% | |
| | .0% | .0% | 66.7% | 33.3% | .0% | | |
| | .0% | .0% | 2.9% | 1.4% | .0% | | |
| Column Total | 64 | 68 | 69 | 71 | 66 | 338 | |
| | 18.9% | 20.1% | 20.4% | 21.0% | 19.5% | 100.0% | |

| Chi-Square | Value | DF | Significance |
|--------------------------|---------|----|--------------|
| Pearson | 7.02811 | 8 | .53360 |
| Likelihood Ratio | 7.70768 | 8 | .46253 |
| Mantel-Haenszel test for | .15092 | 1 | .69765 |

APPENDIX 11. Crosstab Data for Figure 8: % of IDI CD23 Group with Eczema

Y4C26A1 by GP5I23 LOG10 IN-DEPTH CD23 GROUP 20%

GP5I23

| Count Exp Val Row Pct Col Pct | | LE.35 | GT.35 LE | GT.50 LE | GT.60 LE | GT.74 | Row Total |
|----------------------------------------|-----------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|---------------|
| | | 1.00 | .50 2.00 | .60 3.00 | .74 4.00 | 5.00 | |
| Y4C26A1 | 1.00 | 11 7.8 25.6% 18.3% | 7 9.4 16.3% 9.7% | 7 8.3 16.3% 10.9% | 10 8.7 23.3% 14.9% | 8 8.7 18.6% 11.9% | 43 13.0% |
| | 2.00 | 49 51.8 17.2% 81.7% | 65 62.2 22.8% 90.3% | 55 55.3 19.3% 85.9% | 57 57.9 20.0% 85.1% | 59 57.9 20.7% 88.1% | 285 86.4% |
| | 9.00 | 0 .4 .0% .0% | 0 .4 .0% .0% | 2 .4 100.0% 3.1% | 0 .4 .0% .0% | 0 .4 .0% .0% | 2 .6% |
| | Column Total | 60 18.2% | 72 21.8% | 64 19.4% | 67 20.3% | 67 20.3% | 330 100.0% |

| Chi-Square | Value | DF | Significance |
|--------------------------|----------|----|--------------|
| Pearson | 10.99053 | 8 | .20224 |
| Likelihood Ratio | 9.15777 | 8 | .32916 |
| Mantel-Haenszel test for | .05280 | 1 | .81826 |

APPENDIX 12. Crosstab for Figure : % of Cord CD25 Group with Asthma
 Y4MDASMA md diagnosed asthma by GP5C25 CORD CD25 LOG10 GROUPED 20%

| | | GP5C25 | | | | | Row Total |
|----------|---------|---------|---------|---------|----------|---------|--------------|
| Y4MDASMA | Count | LE1.846 | GT1.846 | GT1.915 | GT1.98 L | GT2.071 | |
| | Exp Val | LE1.846 | GT1.846 | GT1.915 | GT1.98 L | GT2.071 | |
| | Row Pct | LE1.915 | LE1.98 | E2.071 | | | |
| | Col Pct | 1.00 | 2.00 | 3.00 | 4.00 | 5.00 | |
| mdasma | 1.00 | 8 | 8 | 7 | 11 | 11 | 45 |
| | | 8.8 | 9.2 | 9.1 | 9.1 | 8.8 | 14.9% |
| | | 17.8% | 17.8% | 15.6% | 24.4% | 24.4% | |
| | | 13.6% | 12.9% | 11.5% | 18.0% | 18.6% | |
| nomdasma | 2.00 | 0 | 0 | 1 | 1 | 0 | 2 |
| | | .4 | .4 | .4 | .4 | .4 | .7% |
| | | .0% | .0% | 50.0% | 50.0% | .0% | |
| | | .0% | .0% | 1.6% | 1.5% | .0% | |
| noasma | 3.00 | 51 | 54 | 53 | 49 | 48 | 255 |
| | | 49.8 | 52.4 | 51.5 | 51.5 | 49.8 | 84.4% |
| | | 20.0% | 21.2% | 20.8% | 19.2% | 18.8% | |
| | | 86.4% | 87.1% | 86.9% | 80.3% | 81.4% | |
| Column | | 59 | 62 | 61 | 61 | 59 | 302 |
| Total | | 19.5% | 20.5% | 20.2% | 20.2% | 19.5% | 100.0% |

| Chi-Square | Value | DF | Significance |
|--------------------------|---------|----|--------------|
| Pearson | 4.93644 | 8 | .76435 |
| Likelihood Ratio | 5.59794 | 8 | .69217 |
| Mantel-Haenszel test for | 1.22442 | 1 | .26850 |

APPENDIX 13. Crosstab for Figure 9: % IDI Group CD25 with Asthma

Y4MDASMA md diagnosed asthma by GP5I25 LOG10 IN-DEPTH CD25 GROUP 20%

GP125

| Y4MDASMA | Count | GP125 | | | | | Row Total |
|----------|---------|---------|---------|----------|----------|-------|--------------|
| | Exp Val | LE 1.69 | GT 1.69 | GT1.81 L | GT1.91 L | | |
| | Row Pct | LE 1.81 | E 1.91 | E 2.02 | | | |
| | Col Pct | 1.00 | 2.00 | 3.00 | 4.00 | 5.00 | |
| mdasma | 1.00 | 9 | 11 | 6 | 15 | 6 | 47 |
| | | 8.6 | 9.6 | 8.9 | 10.2 | 9.6 | 14.6% |
| | | 19.1% | 23.4% | 12.8% | 31.9% | 12.8% | |
| | | 15.3% | 16.7% | 9.8% | 21.4% | 9.1% | |
| nomdasma | 2.00 | 1 | 0 | 0 | 1 | 1 | 3 |
| | | .5 | .6 | .6 | .7 | .6 | .9% |
| | | 33.3% | .0% | .0% | 33.3% | 33.3% | |
| | | 1.7% | .0% | .0% | 1.4% | 1.5% | |
| noasma | 3.00 | 49 | 55 | 55 | 54 | 59 | 272 |
| | | 49.8 | 55.8 | 51.5 | 59.1 | 55.8 | 84.5% |
| | | 18.0% | 20.2% | 20.2% | 19.9% | 21.7% | |
| | | 83.1% | 83.3% | 90.2% | 77.1% | 89.4% | |
| Column | | 59 | 66 | 61 | 70 | 66 | 322 |
| Total | | 18.3% | 20.5% | 18.9% | 21.7% | 20.5% | 100.0% |

| Chi-Square | Value | DF | Significance |
|--------------------------|---------|----|--------------|
| Pearson | 7.63887 | 8 | .46952 |
| Likelihood Ratio | 8.73397 | 8 | .36523 |
| Mantel-Haenszel test for | .22166 | 1 | .63778 |

APPENDIX 14. Crosstab for Figure 10: % of Cord CD25 Group with Hayfever

MDHAY MD DIAG HAYFEVER by GP5C25 CORD CD25 LOG10 GROUPED 20%

| GP5C25 | | | | | | | |
|--------|---------|---------|---------|---------|----------|---------|--------------|
| MDHAY | Count | LE1.846 | GT1.846 | GT1.915 | GT1.98 L | GT2.071 | Row Total |
| | Exp Val | | LE1.915 | LE1.98 | E2.071 | | |
| | Col Pct | 1.00 | 2.00 | 3.00 | 4.00 | 5.00 | |
| NOHAY | .00 | 21 | 29 | 27 | 21 | 25 | 123 |
| | | 22.8 | 26.4 | 24.2 | 25.9 | 23.7 | 44.7% |
| | | 17.1% | 23.6% | 22.0% | 17.1% | 20.3% | |
| | | 41.2% | 49.2% | 50.0% | 36.2% | 47.2% | |
| MDHAY | 1.00 | 24 | 25 | 23 | 28 | 22 | 122 |
| | | 22.6 | 26.2 | 24.0 | 25.7 | 23.5 | 44.4% |
| | | 19.7% | 20.5% | 18.9% | 23.0% | 18.0% | |
| | | 47.1% | 42.4% | 42.6% | 48.3% | 41.5% | |
| MAYBE | 2.00 | 6 | 5 | 4 | 9 | 6 | 30 |
| | | 5.6 | 6.4 | 5.9 | 6.3 | 5.8 | 10.9% |
| | | 20.0% | 16.7% | 13.3% | 30.0% | 20.0% | |
| | | 11.8% | 8.5% | 7.4% | 15.5% | 11.3% | |
| Column | | 51 | 59 | 54 | 58 | 53 | 275 |
| Total | | 18.5% | 21.5% | 19.6% | 21.1% | 19.3% | 100.0% |

| Chi-Square | Value | DF | Significance |
|--------------------------|---------|----|--------------|
| Pearson | 4.32060 | 8 | .82710 |
| Likelihood Ratio | 4.33699 | 8 | .82551 |
| Mantel-Haenszel test for | .10636 | 1 | .74433 |

APPENDIX 15. Crosstab for Figure 10: % of IDI CD25 Group with Hayfever

MDHAY MD DIAG HAYFEVER by GP5I25 LOG10 IN-DEPTH CD25 GROUP 20%

| GP5I25 | | | | | | | |
|--------|---------|---------|---------|----------|----------|-----------|--------|
| MDHAY | Count | LE 1.69 | GT 1.69 | GT1.81 L | GT1.91 L | Row Total | |
| | Exp Val | | LE 1.81 | E 1.91 | E 2.02 | | |
| | Row Pct | | | | | | |
| | Col Pct | 1.00 | 2.00 | 3.00 | 4.00 | | 5.00 |
| | .00 | 25 | 21 | 21 | 30 | 24 | 121 |
| NOHAY | | 21.5 | 25.3 | 21.5 | 27.0 | 25.7 | 42.2% |
| | | 20.7% | 17.4% | 17.4% | 24.8% | 19.8% | |
| | | 49.0% | 35.0% | 41.2% | 46.9% | 39.3% | |
| MDHAY | 1.00 | 17 | 34 | 20 | 29 | 29 | 129 |
| | | 22.9 | 27.0 | 22.9 | 28.8 | 27.4 | 44.9% |
| | | 13.2% | 26.4% | 15.5% | 22.5% | 22.5% | |
| MAYBE | 2.00 | 9 | 5 | 10 | 5 | 8 | 37 |
| | | 6.6 | 7.7 | 6.6 | 8.3 | 7.9 | 12.9% |
| | | 24.3% | 13.5% | 27.0% | 13.5% | 21.6% | |
| | | 17.6% | 8.3% | 19.6% | 7.8% | 13.1% | |
| | Column | 51 | 60 | 51 | 64 | 61 | 287 |
| | Total | 17.8% | 20.9% | 17.8% | 22.3% | 21.3% | 100.0% |

| Chi-Square | Value | DF | Significance |
|--------------------------|----------|----|--------------|
| Pearson | 10.52154 | 8 | .23031 |
| Likelihood Ratio | 10.56558 | 8 | .22755 |
| Mantel-Haenszel test for | .01468 | 1 | .90357 |

APPENDIX 16. Crosstab for Figure 11: % of Cord CD25 Group with Eczema

Eczema (Y4C26A1) by GP5C25 CORD CD25 LOG10 GROUPED 20%

GP5C25

| Y4C26A1 | Count | LE1.846 | GT1.846 | GT1.915 | GT1.98 L | GT2.071 | Row Total |
|-----------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|---------------|--------------|
| | Exp Val | | LE1.915 | LE1.98 | E2.071 | | |
| | Row Pct | 1.00 | 2.00 | 3.00 | 4.00 | 5.00 | |
| | Col Pct | | | | | | |
| 1.00 | 8 8.2 19.0% 13.6% | 9 8.5 21.4% 14.8% | 9 8.5 21.4% 14.8% | 11 8.6 26.2% 17.7% | 5 8.3 11.9% 8.3% | 42 13.9% | |
| 2.00 | 51 50.2 19.8% 86.4% | 51 51.9 19.8% 83.6% | 52 51.9 20.2% 85.2% | 50 52.8 19.4% 80.6% | 54 51.1 20.9% 90.0% | 258 85.1% | |
| 9.00 | 0 .6 .0% .0% | 1 .6 33.3% 1.6% | 0 .6 .0% .0% | 1 .6 33.3% 1.6% | 1 .6 33.3% 1.7% | 3 1.0% | |
| Column Total | 59 19.5% | 61 20.1% | 61 20.1% | 62 20.5% | 60 19.8% | 303 100.0% | |

| Chi-Square | Value | DF | Significance |
|--------------------------|---------|----|--------------|
| Pearson | 4.38066 | 8 | .82125 |
| Likelihood Ratio | 5.60394 | 8 | .69150 |
| Mantel-Haenszel test for | .89129 | 1 | .34513 |

APPENDIX 17. Crosstab for Figure 11: % of IDI CD25 Group with Eczema

ECZEMA (Y4C26A1) by GP5I25 LOG10 IN-DEPTH CD25 GROUP 20%

GP5I25

| Y4C26A1 | Count Exp Val Row Pct Col Pct | GP5I25 | | | | | Row Total |
|-----------------|----------------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|-------------|---------------|
| | | LE 1.69 | GT 1.69 | GT1.81 L | GT1.91 L | | |
| | | LE 1.81 | E 1.91 | E 2.02 | | | |
| | | 1.00 | 2.00 | 3.00 | 4.00 | 5.00 | |
| 1.00 | 5 7.7 11.9% 8.5% | 9 8.7 21.4% 13.4% | 8 7.9 19.0% 13.1% | 7 9.1 16.7% 10.0% | 13 8.6 31.0% 19.7% | | 42 13.0% |
| 2.00 | 54 50.8 19.4% 91.5% | 57 57.7 20.5% 85.1% | 51 52.5 18.3% 83.6% | 63 60.2 22.7% 90.0% | 53 56.8 19.1% 80.3% | | 278 86.1% |
| 9.00 | 0 .5 .0% .0% | 1 .6 33.3% 1.5% | 2 .6 66.7% 3.3% | 0 .7 .0% .0% | 0 .6 .0% .0% | | 3 .9% |
| Column Total | | 59 18.3% | 67 20.7% | 61 18.9% | 70 21.7% | 66 20.4% | 323 100.0% |

| Chi-Square | Value | DF | Significance |
|--------------------------|----------|----|--------------|
| Pearson | 10.00289 | 8 | .26482 |
| Likelihood Ratio | 10.19130 | 8 | .25186 |
| Mantel-Haenszel test for | 1.06780 | 1 | .30144 |

APPENDIX 18.

DATA FOR FIGURE 12

Summaries of LMIC23 LOG10 IN-DEPTH MEAN CD23
 By levels of GP5I25 LOG10 IN-DEPTH CD25 GROUP 20%

| Variable | Value | Label | Mean | Std Dev | Cases |
|-----------------------|-------|-----------------|-------|---------|-------|
| For Entire Population | | | .5335 | .2860 | 312 |
| GP5I25 | 1.00 | LE 1.69 | .5181 | .3387 | 55 |
| GP5I25 | 2.00 | GT 1.69 LE 1.81 | .4982 | .2134 | 67 |
| GP5I25 | 3.00 | GT 1.81 LE 1.91 | .4428 | .3847 | 61 |
| GP5I25 | 4.00 | GT 1.91 LE 2.02 | .6213 | .2157 | 69 |
| GP5I25 | 5.00 | GT 2.02 | .5781 | .2235 | 60 |

Analysis of Variance

| Source | Sum of Squares | D.F. | Mean Square | F | Sig. |
|--------------------------|----------------|------|-------------|--------|-------|
| Between Groups | 1.2502 | 4 | .3126 | 3.9664 | .0037 |
| Linearity | .4025 | 1 | .4025 | 5.1076 | .0245 |
| Deviation from Linearity | .8477 | 3 | .2826 | 3.5360 | .0142 |

R = .1258 R Squared = .0158

| | | | |
|---------------|---------|-----|-------|
| Within Groups | 24.1917 | 307 | .0788 |
|---------------|---------|-----|-------|

Eta = .2217 Eta Squared = .0491

APPENDIX 19. DATA FOR TABLE 3: CD23 VALUES

Variable LOG 10 CORD MEAN CD23 By Variable INDSEX (GENDER)

Analysis of Variance

| Source | D.F. | Sum of Squares | Mean Squares | F Ratio | F Prob. |
|-------------------|------|-------------------|-----------------|------------|------------|
| Between Groups | 1 | .0531 | .0531 | .8092 | .3690 |
| Within Groups | 338 | 22.1869 | .0656 | | |
| Total | 339 | 22.2400 | | | |

- - - - - O N E W A Y - - - - -

Group 1 = Male

Group 2 = Female

| Group | Count | Mean | Standard Deviation | Standard Error | 95 Conf Int for | Mean |
|----------------------|-------|-------|-----------------------|-------------------|--------------------|-------|
| Grp 1 | 164 | .5844 | .2543 | .0199 | .5452 To | .6236 |
| Grp 2 | 176 | .6094 | .2579 | .0194 | .5711 To | .6478 |
| Total | 340 | .5974 | .2561 | .0139 | .5700 To | .6247 |
| Fixed Effects Model | | | .2562 | .0139 | .5700 To | .6247 |
| Random Effects Model | | | | .0139 | .4208 To | .7739 |

APPENDIX 20. DATA FOR TABLE 3: CD25 VALUES

Variable Log10 MCD25 By Variable INDSEX (Gender)

Analysis of Variance

| Source | D.F. | Sum of Squares | Mean Squares | F Ratio | F Prob. |
|----------------|------|----------------|--------------|---------|---------|
| Between Groups | 1 | .0021 | .0021 | .1088 | .7418 |
| Within Groups | 302 | 5.8598 | .0194 | | |
| Total | 303 | 5.8619 | | | |

- - - - - O N E W A Y - - - - -

Group (Grp) 1 = Male
 Group (Grp) 2 = Female

| Grp | Count | Mean | Standard Deviation | Standard Error | 95 Conf Int for Mean |
|----------------|-------|--------|--------------------|----------------|----------------------|
| 1 | 147 | 1.9520 | .1440 | .0119 | 1.9285 To 1.9755 |
| 2 | 157 | 1.9467 | .1347 | .0108 | 1.9255 To 1.9680 |
| Tot | 304 | 1.9493 | .1391 | .0080 | 1.9336 To 1.9650 |
| Fixed Effects | | | | | |
| Model | | | .1393 | .0080 | 1.9336 To 1.9650 |
| Random Effects | | | | | |
| Model | | | | .0080 | 1.8478 To 2.0508 |

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